ERRATA

The following errors should be corrected in the report, "Imprinting Salmon and Steelhead Trout for Homing", by Emil Slatick, Anthony J. Novotny, and Lyle G. Gilbreath, dated January 1979:

1. Page 6, Table 2, Test #4 Brand position--now reads, "RAL", and it should read RA⌐.

2. Page 12, Table 6, Control #2, Brand position--now reads, "LA≍, and it should read LA)(.

3. Page 14, Table 7, Test #1, Treatment--now reads, "(raceways)", and it should read (tank).

4. Page 22, line 5--now reads, "Dam in two loads for marking on 19 and 20 May 1976", and it should Dam in two loads for marking on 19 and 20 May 1976, and transported and released below Bonneville Dam.

STUDY OF DISEASE AND PHYSIOLOGY IN THE 1978 HOMING STUDY HATCHERY STOCKS-A SUPPLEMENT TO: "IMPRINTING SALMON AND STEELHEAD TROUT FOR HOMING" BY SLATICK, NOVOTNY, AND GILBREATH, JANUARY 1979

by

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INTRODUCTION

The main functions of the National Marine Fisheries Service (NMFS) Aquaculture Task biologists and contractual scientists involved in the 1978 homing studies were primarily a surveillance of fish physiology, disease, and relative survival during culture in marine net-pens, to determine if there were any unusual factors that might effect imprinting and homing behavior. The studies were conducted with little background knowledge of the implications of disease and physiology on imprinting and homing in salmonids. Hatcheries and stocks sampled are listed in Table 1.

The data collected from random samples were as follows:

I. PHYSIOLOGY.

A. Gill Na+-K+ ATPase activities were measured at the time of release, with the exception of the Willard Hatchery coho salmon and Tucannon Hatchery steelhead, where freshwater Na+-K^+ ATPase profiles were conducted throughout the spring. Abnormally low values could be indications that the fish were either in pre- or post-smelt condition, or had been stressed in some way.

B. Plasma electrolytes. Low values of Na or Cl could indicate immediate problems of osmoregulation when the fish were introduced to seawater; high values may iandicate some dehydration due to stress. Increases in K levels can sometimes indicate nitrogen supersaturation stresses.

C. Hematocrits and hemoglobins. Values below or above normal can indicate possible conditions of anemia or dehydration, or reflect nutritional status, disease conditions,or both.

TABLE l.--Hatcheries and stocks sampled in the 1978 homing studies.

Hatchery	Species	Pathology tag nos.	Date arrived at Manchester	Date of viral assay	Date of blood sampling	Date transferred to seawater pens	AtPase profile
Carson	Coho	4401-4460	4/25/78	4/25/78	4/26/78	4/27/78	No
Willard	Coho	4661-4700 4351-4370	6/ 8/78	6/12/78	6/12/78	6/10/78	Yes
Kooskia	Spring chinook	4001-4060	4/25/78	4/25/78	4/25/78	4/27/78	No
Dworshak	Steelhead	4061-4120	4/25/78	4/26/78	4/26/78	4/27/78	No
Leavenworth (Chelan)	Steelhead	3901-3960	5/ 2/78	5/3/78	5/ 4/78	5/ 4/78	No
Winthrop (Wells)	Steelhead	4501-4560	5/ 2/78	5/5/78	5/ 9/78	5/ 4/78	No
Tucannon	Steelhead	4601-4660	5/15/78	5/16/78	5/17/78	5/17/78	Yes

II. DISEASES.

A. Incidences of diseases based on hatchery records describing the culture and treatment of fish.

B. Random sampling of hatchery populations to determine:

1. The extent of latent bacterial kidney disease (BKD) as determined by indirect flourescent antibody techniques.

2. The presence (or absence) of certain pathogenic viruses.

3. A determination of significant lesions, abnormalities, or pathology in gill, eye, liver, and kidney tissue.

The presence and extent of the above disease organisms or lesions may have a detrimental effect upon the physiological mechanisms involved in imprinting and homing behavior.

III. SURVIVAL DURING CULTURE IN SEA-PENS.

A. Periodic assessment of survival and growth.

B. Necropsy of mortalities.

C. Assessment of the major causes of mortality.

Culturing subsamples of these hatchery test groups in net-pens in seawater is an artifical situation and is recognized as such. Lower survival may not be indicative of what is occurring in nature, as: (1) the fish are transferred directly from fresh to 30°/oo seawater without conditioning in estuarine water (as presumably might be the case in nature); (2) they are fed an artificial diet; and (3) they are contained in net-pens and stressed by frequent (monthly) measurement activities.

Nevertheless, one can assume that if the survival in the net-pens was high, the fish should be able to withstand the normal transition rigors in the wild, and that the tests may be a relative measure of seawater adaptability between treatments or stocks.

?lETHODS AND MATERIALS

VIRAL ASSAYS

Liver, spleen, and kidney were sampled from 60 fish in each test group, pooled in 12 tubes of 5 fish each, and screened by a private laboratory (Rangen Research Laboratories) for viruses, See Appendix A.

HLSTOPATHOLOGY

Sixty individually numbered fish of each test group in the homing study were preserved in successive fixatives and shipped to a private laboratory. Gill, liver, eye, and kidney tissues were sectioned and examined for any lesions, pathology, or abnormalities by a veterinary pathologist. See Appendix B.

EARLY LIFE HISTORY

Wherever possible, pertinent data concerning the culture and treatment of the hatchery groups were collected from the hatchery managers. These data are presented in Table 2.

SAMPLING

The sampling of fish from the hatchery stocks for health profiles was based on a combination of statistics and economics. The random sample of 60 fish from populations ranging as high as 100,000 or more was based on the work of Ossiander and Wedemeyer (1973). A single disease incidence of 5% or

Table 2.--Available disease and life history data of the homing study hatchery juveniles.

Hatchery	Stock	Agency[1]	Species	Date egg take	Date ponded	Feed[2]	Water source	Water temp °F	Percent mortality (all causes)	Size at release (no./lb.)	Date released (1978)	Date transferred to Manchester (1978)	Diseases[3]	Disease treatment[4]
Tucannon	Skamania	WDG	Summer Steelhead	1976	March 1976	OMP Clark's Silver Cup	----	33-70	43.0	7.2	May 15	May 15	Costiasis Fungus BGD Columnaris Ichthyophthirius (June, 1979)	Malachite green Formalin Sulfas TM-50 (daily; last 2 weeks prior to release)
Dworshak	Dworshak	USFWS	Steelhead	----	----	OMP	River	40-60	----	8.0	April 25	April 25	External parasites	Formalin
Chelan (Leavenworth)	Chelan	WDG	Summer Steelhead	1976	----	Clark's Salmon Clark's Trout Silver Cup Salmon Silver Cup Trout OMP	----	50-58	37.5	7.0	May 3	May 2	Vitamin C deficiency External parasites Unidentified systemic infection BGD	TM-50 Sulmet
Wells (Winthrop)	Wells	WDG	Steelhead	1977	1977	OMP Clark's Silver Cup	Well and River	50	28.0	5.5	May 6	May 5	----	----
Kooskia	Kooskia	USFWS	Spring Chinook	----	----	OMP Abernathy dry	Well and River	32-60	----	17.0	April 25	April 25	BKD Ichthyophthirius	Formalin
Carson	Carson	USFWS	Coho	----	1977	OMP Abernathy dry	Well	44	----	----	April	April 25	Furunculosis	TM-50 Roccal
Willard	Willard	USFWS	Coho	1976	1977	OMP	Well and River	44	16.0	21.6	June 8	June 8	CWD BKD Furunculosis	TM-50

1/ WDG - Washington Department of Game, USFWS - United States Fish and Wildlife Service. 2/ OMP - Oregon Moist Pellet.

3/ BGD - bacterial gill disease, BKD - bacterial kidney disease, CWD - cold water disease. 4/ TM-50 - oxytetracycline (dietary).

greater can be detected, provided the detection method is accurate. Subsamples of at least 60 fish were taken at the hatchery, or were drawn from the transported population for the health survey and held in circular tanks with running Beaver Creek water at ambient creek temperatures. When the average fish size was small, two subsamples of 60 fish each were required to provide sufficient material (blood and tissue) for the analyses. In most cases, the tissue and blood samples were collected within 24 hours after arrival at Manchester. However, some stocks were held as long as 6 days prior to processing. The fish from some of the hatcheries (primarily fall chinook salmon) were small. To obtain the required volumes of blood and tissue samples,we discarded any undersized fish, but still maintained a 60 fish sample size.

The fish subsampled for viral assays were individually weighed and measured. Fish that were subsampled for hematology and BKD were measured, but not weighed. Homing study fish preserved for histological sectioning were identified by numbered tags. Collected data on all parameters are available on an individual fish basis.

BLOOD SAMPLE COLLECTION

The fish were lightly anesthetized in aerated 1:20,000 MS-222 solutions. In most cases, blood was sampled from the caudal arch via 1 cc heparinized syringe and 25 gauge hypodermic needle. Small fish were bled by severing the caudal peduncle and collecting the blood in heparinized CAPILLARY tubes.

Whole blood smears were collected on microscope slides, labelled, air dried, fixed and stained in Diff-Quick," oven dried overnight at 45'C, and permanently mounted for future reference. The final data for hematocrits and hemoglobins were frequently based on sample sizes of less than 63 due to capillary tube breakage.

1/ Reference to trade names does not imply endorsement by National Marine Fisheries Service, NOAA.

HEMATOCRITS

Blood samples taken for hematocrits (packed cell volume) were centrifuged in microhematocrit tubes for 3 minutes in a Clay-Adams Autocrit II (Snieszko 1960).

HEMOGLOBINS

Blood samples for hemoglobin determination were either read directly with an A-O hemoglobinometer or collected in 20ul capillary tubes to determine hemoglobin concentration by the calorimetric method described by Bauer (1970).

SAMPLING FOR LATENT BACTERIAL KIDNEY DISEASE (BKD)

The sensitive and highly specific indirect flourescent antibody technique (IFAT) was used to diagnose latent BKD in hatchery populations.

The individually indentified fish were opened ventrally and the kidney exposed. Thin smears of anterior and posterior kidney tissue were made on multi-spot slides after piercing the kidney with a sterile inoculation loop. The slides were air-dried and fixed in reagent grade acetone for 10 minutes. The acetone fixed slides were stored at $-20^{o}C$ until they were examined. Prior to the sampling season, 40 positive control slides were prepared in the same manner and stored at $-20^{o}C$. The control slides were prepared from a clean kidney lesion from a spring chinook salmon from Carson National Hatchery that was tested and confirmed to have high numbers of pure BKD organisms.

The IFAT for BKD was originally described by Bullock and Stuckey (1975) and later modified by G. W. Camenisch of the U.S. Fish and Wildlife Service (FWS), Eastern Fish Disease Laboratory (See Appendix C for the complete procedure).

Basically this diagnostic procedure employs the following steps:

1. Application of specific BKD rabbit antisera to the unknown smear of kidney material.

2. Application of goat anti-rabbit IgG fluorescein conjugate to the unknown smear. The specific antibody in the first antisera application will attach to any BKD organisms present in the unknown kidney smear. The second antisera application will then attach to the rabbit antibody (IgG), and the fluorescein serves as a label for the BKD organisms when exposed to flourescent microscopy.

3. The control slide was scanned first to insure that all procedures went normally, and the intensity of flourescence in the BKD control was subjectively noted. Only those organisms that were recognized as having the typical FA-BKD appearance were counted as positive. Any suspect organisms or probable debris were passed over as artifacts. Counts of the number of BKD organisms encountered in the first 150 microscope fields (mfs) were recorded. Samples were counted as positive if only one BKD organism was found. Both anterior and posterior kidney were examined and only those fish that had both kidney samples free of BKD organisms were classed as "negative".

NECROPSIES

All of the mortalities in the saltwater pens were collected daily. Each fish was opened asceptically from the vent; external and internal lesions were noted and the procedures for culturing vibriosis and other gram negative bacteria (Novotny, Harrell, and Nyegaard 1975) were followed.

The postmortems were classified as follows:

1. negative (cause not determined).
2. BKD (from lesions).
3. *Vibrio anguillarum*: serotypes 775, 1669, or 7244.
4. *Vibrio* sp.
5. ERM (enteric redmouth)
6. Furunculosis
7. *Aeromonas hydrophilia* (ex *liquefaciens*)

GILL NA^{+}-K^{+} ATPASE

Since the phenomenon of elevation in gill sodium, potassium stimulated ATPase (Na+-K^{+}ATPase) activity was first reported to be associated with parr smolt transformation in coho salmon, 0. kisutch, (Zaugg and McLain 1970) numerous experiments have been conducted to verify these results and extend observations to other species. As a result, it has been conclusively shown that the rise in gill Na+-K+ ATPase activity is one of the many physiological changes which occur at the time salmonids demonstrate migratory behavior and an increased ability to tolerate seawater.

COLLECTION AND STORAGE OF GILL FILAMENTS

During 1978, selected stocks of coho and chinook salmon, 0. tshawvtscha, and steelhead, Salmo gairdneri, being reared for release at state and federal hatcheries in the Columbia River drainage were monitored for changes in gill Na+-K+ ATPase activities in an attempt to evaluate the state of smoltification at release.

In addition, where possible through tag identification, the relationship was determined between state of smoltification at release and length of migration time from the hatchery to the estuary.

At approximately 2-week intervals during the spring and summer of 1978, 30 fish were removed by dip net from representative ponds or raceways at Willard and Tucannon Hatcheries. Carson, Winthrop (Wells), Leavenworth (Chelan), Kooskia, and Dworshak Hatcheries were sampled only at release. The fish were grouped into ten groups of three fish each (approximately according to size)and they were killed by a blow on the head. After fork lengths and weights were determined, tails were severed to allow bleeding and approximately equal quantities of gill filaments were removed from the gill arches of each

of the three fish in the group (total weight of gill filaments-O.1 to 0.2 g) and placed into a vial to which was then added 1 ml of a solution (labeled SEI) containing 0.3M sucrose, 0.2H Na2 EDTA, and O.lM imidazole, pH 7.0. The samples of filaments in SE1 were kept cold (on ice) until all 30 fish had been processed, at which time they were frozen on dry ice. The samples were later transferred to a freezer at -23°C where they were stored until processed for ATPase activities (stable up to at least 6 weeks). The analytical procedure for measuring ATPase activity is presented in Appendix D.

THE LIFE HISTORY OF HATCHERY JUVENILES

Husbandry techniques, disease, and environmental history may have deleterious effects on fish health and smolt quality (Wedemeyer, et al. 1979, Folmar and Dickhoff 1979). Many chemotherapeutic compounds used in the treatment of parasitic and bacterial diseases of fish may affect smoltification (Lorz and McPherson 1976). Subclinical infections may be exacerbated by the stress of saltwater entry.

The information (Table 2) was obtained from hatchery management and is self-explanatory. Where Information was not obtained, the entries have been left blank.

I. STEELHEAD

A. GILL ENZYME ANALYSES

1. TUCANNON HATCHERY (PATHOLOGY SAMPLE NUMBERS 4601-4660).

Summer run steelhead from the Tucannon Hatchery (Pond 1) showed increases in Na+-K+ ATPase activities through 8 May (Figure 1). Although the average activities dropped somewhat on 22 May and 5 June,

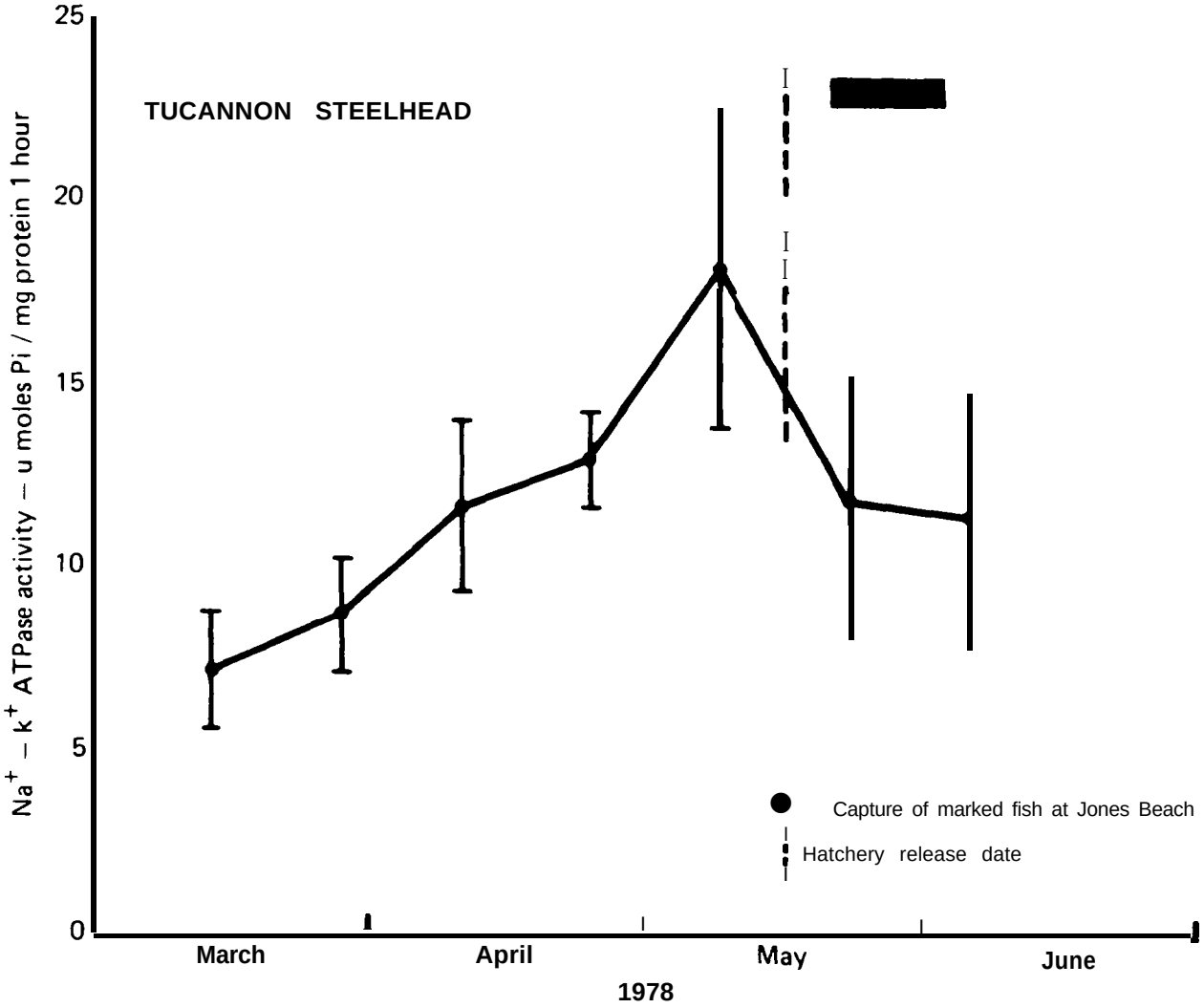


FIGURE 1. Gill Na+ -K+ ATPase enzyme activity in Tucannon steelhead sampled at the hatchery. Enzyme activity was measured at approximately 2-week intervals from mid-March through early June. The data shown for each sampling period are the mean and variance of 10 analyses from 30 fish (pooled- 3 fish/analysis).

the majority of these fish had elevated values. A few had either not smolted or had reverted early, having activities in the 6 to 7 range, resulting in a depression of the average activity. This group of fish (coded tag, W-O-Y-R) was released below Bonneville Dam on 17 May, slightly after the peak of the gill Na+-K+ ATPase activity and moved rapidly through a recapture area in the Columbia River estuary at Jones Beach where tagged fish were caught only from 20 May to 2 June (Figure 1). Although the activity was declining at release, the activity of fish transferred to seawater increased (Table 3). Fish held at the hatchery and sampled on 22 May just after release of the main group averaged 19.5 cm fork length and weighed 65.9 g.

These fish were all sampled by anesthetizing with MS-222, clipping the necessary amount of filament from one or two gill arches, then returning the fish to the pond. Only about 10% of the 210 animals tested failed to survive.

2. DWORSHAK HATCHERY (PATHOLOGY SAMPLE NUMBERS 4061- 4120)

Steelhead were sampled once at Dworshak National Fish Hatchery on 24 April from pond 42, System II, and generally appeared to be Parr. The average gill Na+-K+ ATPase activity was 5.0 + 1.1, ranging from 3.5 to 7.2. Only one group of 3 fish out of the 10 groups on which the enzyme activity was determined had a high enough value (7.2) to suggest even initial stages of smolt transformation. Lack of physical and biological signs of smoltification were undoubtedly due to water temperature which had been ranging from 13^{o} to $15^{o}C$, and the upper limit which will permit smolting in steelhead is about $12^{o}C$ (Zaugg, et al. 1972). The post-seawater Na^{+}-K^{+} ATPase activity increased dramatically (Table 3).

TABLE 3.--Summary data of viral assays, BKD analyses, ATPase analyses, hematological analyses, plasma electrolytes, and survival in the seawater pens of the 1978 homing study of fish.

Stock and species	Specimen nos.	Viral assays (results)	% of latent BKD detectible in the kidney by FAT				10 day post seawater entry gill ATPase activity-umoles pi/mg pr/h				Freshwater gill ATPase activity-umole pi/mg pr/h		Hematological data (taken at Manchester upon arrival)		Plasma electrolytes Meq/l								
															Na			Cl			K		
			Anterior	Posterior	Both	Either/ both	Min.	Max.	X	SD	Date	Activity X	Mean Hematocrit value (%)	Mean Hemoglobin g/100 ml	n	X	SD	n	X	SD	n	X	SD
Dworshak steelhead	4061-4120	IPN-confirmed	10.0	5.0	1.7	16.7	14.8	23.0	18.4	2.6	4-24-78	5.0	52.4	11.3	59	166.4	7.8	57	133.3	9.1	59	1.0	0.8
Leavenworth-Chelan steelhead	3901-3960	Negative	13.3	16.7	56.7	86.7	14.2	28.6	19.5	4.3	5- 3-78	7.5	43.3	8.9	60	165.9	14.8	58	130.9	17.2	60	1.1	0.8
Winthrop-Wells steelhead	4501-4560	IPN-confirmed	16.7	20.0	46.7	83.4	7.1	13.7	10.9	2.8	5- 3-78	17.0	55.6	11.4	58	150.3	11.1	58	107.9	20.6	58	2.5	2.6
Tucannon steelhead	4601-4660	Negative	8.3	3.3	10.0	21.6	10.9	23.4	17.6	4.6	5- 8-78 5-22-78	18.2 11.7	48.5	9.7	60	159.5	9.5	59	131.6	6.5	60	2.4	2.6
Kooskia spring chinook	4001-4060	IPN-confirmed	11.7	8.3	70.0	90.0	17.0	35.0	22.6	5.0	4-24-78	18.1	39.1	6.6	37	114.0	19.2	31	104.1	7.9	not conducted		
Willard coho I	K01-K60	Not assayed	6.7	3.3	5.0	15.0	-	-	*16.4	1.5	5- 7-78	12.8	42.5	6.4	-	143.8	-	-	120.4	-	-	7.5	-
Willard coho II	N01-N60	Negative	6.7	3.3	6.7	16.7	-	-	*14.6	1.5	5-22-78	15.0	45.7	7.2	-	154.3	-	-	123.7	-	-	6.9	-
Willard coho III	4661-4700 4351-4370	IPN-confirmed	11.9	20.3	8.5	40.7	17.4	25.3	22.5	2.9	6- 8-78	8.9	42.3	8.4	52	144.3	10.9	43	115.4	7.6	52	5.8	2.7
Carson coho	4401-4460	IPN-confirmed	0	1.7	3.3	5.0	20.7	33.6	28.2	4.0	4-27-78	11.1	32.9	4.7	39	139.4	11.3	33	124.4	11.3	39	4.1	1.4
Expected ranges for clinically healthy rainbow trout													24-43	5.4-9.3	130-170			111-155			1.4-6.0		

* 8 days

TABLE . 3.--Continued.

Stock and species	Seawater survival (in net-pens) N (start)	Survival (%) 30 day post entry	Survival (%) as of 9-24-78	N (end)	% Survival. to termination (October-1978)	Causes of mortality during seawater culture Negative for pathogenic bacteria	% BKD	Vibrio (%)	ERM (%)	Furunculosis (%)	Aeromonas hydeophila (%)
Dworshak steelhead	178	46.4	10.8	7	3.9	73.4	0	15.6	7.8	3.1	0
Leavenworth-Chelan steelhead	180	80.0	20.0	28	15.6	44.9	0	55.1	0	0	0
Winthrop-Wells steelhead	179	10.5	0	4	2.2	2.0**	0	1.3	0	0	0.7
Tucannon steelhead	330	85.8	44.5	120	36.4	30.5	0	69.5	0	0	0
Kooskia spring chinook	180	98.8	50.0	66	36.7	36.8	10.5	42.1	5.3	5.3	0
Willard coho I	300	95.0	36.7	103	34.3	19.4	5.6	75.0	0	0	0
Willard coho II	300	96.0	46.3	124	41.3	51.4	2.9	45.7.	0	0	0
Willard coho III	300	97.0	43.3	118	39.3	34.2	7.9	57.9	0	0	0
Carson coho	180.	98.2	88.0	83	46.1	52.4	4.8	42.9	0	0	0

**96X were classed as osmoregulatory stress.

3. CHELAN HATCHERY (TRANSFERRED To LEAVESWORTH HATCHERY) (PATHOLOGY NUMBERS 3901-3960).

Chelan Hatchery steelhead sampled at Leavenworth Hatchery on 3 May gave and average gill Na+-K+ ATPase activity of 7.5 + 1.8, with values ranging from 4.2 to 11.1. Without having previous samples, it is difficult to determine the degree of smoltification from ATPase activities. However, values of 4 to 6 (3 groups) probably reflected little or no smolting. Values of 7 and 8 (6 groups) may have indicated some degree of transformation, while one group with 11 may have been completely transformed. General appearance suggested that most fish were in a transitional state. Average fork length was 21.0 cm and weight 79.4 g.

4. WELLS HATCHERY (TRANSFERRED TO WINTHROP HATCHERY) STEELHEAD (PATHOLOGY NUMBERS 4501-4560).

Na+-K+ ATPase activities were determined on representative samples of Wells Hatchery steelhead, sampled at Winthrop Hatchery on 3 May. These fish had an average gill Na+-K+ ATPase activity of 17.0 ± 5.1, with values ranging from 11.6 to 26.6. The sampled fish averaged 22.5 cm fork length and weighed 102.2 g and were judged to be in a good smolted condition. However, this was the only group of fish sampled in which the gill Na^+-K^+ ATPase value declined significantly after transfer to seawater.

B. PLASMA ELECTROLYTES.

Sodium, potassium, and chloride ion levels in plasma were determined for fish near the time of release. This may be the first collected data on plasma electrolyte levels for steelhead; the published literature contains data for rainbow trout only.

A compilation of data on rainbow trout by Miles and Smith (1968) suggests expected values (in fresh water) of 130-170 meq (milliequivalents) /l for Na; 1.4-6.0 meq/l for K; and 115-155 meq/l for Cl. Hickman, et al. (1964) reported plasma Na ranges of 152-172 meq/l; Cl ranges of 111 to 145 meq/l; and K ranges of 3.5 to 4.2 meq/l at temperatures of 6 and 16'C.

Combining these reported ranges, we could expect the following ranges to be normal or near normal: Na+ 130 to 172 meq/l; Cl- 111 to 155 meq/l; and K+ 1.4 to 6.0 meq/l.

1. TUCANNON HATCHERY.

The summary data for plasma electrolytes for the homing stocks are listed in Table 3. The mean values for Na, K, and Cl of the Tucannon steelhead fall within the expected ranges for rainbow trout. However, 43.3% of the Tucannon Hatchery samples were below the minimum range reported for K in rainbow trout.

2. DWORSHAK HATCHERY.

The mean plasma Na values for Dworshak Hatchery steelhead (Table 3). were considerably higher than Tucannon Hatchery steelhead (166.4 meq/l); mean Cl values were similar to the Tucannon fish (133.3 meq/l), but both are within the expected range for rainbow trout. The mean plasma K values were considerably lower (1.0 meq/l) than the Tucannon steelhead, and definitely lower than the lowest expected values for rainbow trout. There were no samples with K levels above the maximum expected for rainbow trout and 76.2% were below the minimum expected value for rainbow trout. The latter could be stress related or since the fish were sampled at the hatchery, a general hypokalemic (low blood K+) condition.

3. CHELAN HATCHERY.

The plasma electrolytes of the Chelan Hatchery steelhead were quite similar to the Dworshak Hatchery fish (Table 3). The mean sodium and chloride values were near the upper limits reported for clinically healthy trout. The potassium values were low, as 63.3% of the samples were below the minimum for clinically healthy rainbow trout, again indicating possible stress factors. None of the potassium values were above the maximum reported for rainbow trout.

4. WELLS-WINTHROP HATCHERY.

There were noticeable differences in the plasma electrolytes of the Wells-Winthrop steelhead when compared to the other steelhead stocks in these studies (Table 3). First, the mean plasma K levels were almost identical to the Tucannon Hatchery stock (whichwere within the lower ranges expected in healthy rainbow trout), but again with a large variance; 41.3% were below the minimum expected value, and 8.6% were above the maximum expected value. The mean Na value of 150.3 meq/l is well within the expected range for healthy rainbow trout, but the mean Cl value fell below the minimum expected value for healthy rainbow trout. The mean Na and Cl values were the lowest of any of the steelhead stocks surveyed and statistically they were significantly different from the mean values of the other steelhead stocks. There was a high mortality in transit to Manchester and even though the survivors were rested for 7 days prior to sampling, these lower comparative values may be a reflection of that or some other stress factor.

C. HEMATOLOGY

There is no published information available on hematological values for normal wild or hatchery reared steelhead. Therefore, we can only make comparisons to the wide range of hematological values that appear in the literature for rainbow trout.

Houston and DeWilde (1968) reported the means and variances of hematocrits and hemoglobins for rainbow trout as 31.6 + 0.3% and 7.4 ± 0.15 g hemoglobin/100 ml blood, respectively. Wedemeyer and Yasutake (1977) point out the clinically healthy rainbow trout can be expected to have hematocrits ranging from 24 to 43%M and hemoglobin values ranging from 5.4 to 9.3 g/100 ml blood. Bamhart (1969) sampled several strains of rainbow trout fed two different diets and found mean hematocrit values ranging from 28.2 to 31.7%, with individual hematocrits ranging from 11 to 44%, and mean hemoglobin values ranging from 6.5 to 7.7 g/100 ml blood, with individual hemoglobins ranging from 2.2 to 13.0 g/100 ml blood.

McCarthy et al. (1973) studied the Kamloops variety of rainbow trout and found mean hematocrits of 39.5% (range: 30-49%) and mean hemoglobin values of 7.5 g/100 ml blood (range: 5.2-12.9 g/100 ml). The 96% percentile ranges were: Hct - 24.0-43.0; Hb - 5.4-9.3.

Wedemeyer and Nelson (1975) reported simlliar values for the Shasta strain of rainbow trout. The means and percentile range estimates were: Hct - 34.1% (24.0-43.0); Hb - 7.6 g/100 blood (5.4-9.3).

Snieszko (1960) studied different size groups of rainbow trout, and found that fish averaging 142 mm long had mean hematocrits of 45.3%; and, fish averaging 235 mm in length had mean hematocrits of 53%. Approximately

10% of the smaller trout and 73% of the larger trout had hematocrits of 50% or more. The mean hemoglobin value of the larger fish was 8.7 g/100 ml. Most steelhead smolts would be between these two size groups.

1. TUCANNON HATCHERY.

The summarized data of the hematocrit and hemoglobin values for the Tucannon steelhead are presented in Figure 2. The mean hematocrit (48.6%) and hemoglobin (9.9 g/100 ml blood) values were higher than the maximum expected values for rainbow trout reported by Wedemeyer and Yasutake (1977); 72.2% of the hematocrit values were above 43%, and 55.0% of the hemoglobin values were above 9.3 g/100 ml blood.

The blood samples were taken 48 hours after they arrived at Manchester (see Table 1) and there was no great deviation in water temperatures. Therefore, these high mean values should not be due to transport stress. Other evidence suggests that the fish were reasonably healthy, and these data may reflect normal values for steelhead, or they may be related to smoltification.

2. DWORSHAK HATCHERY.

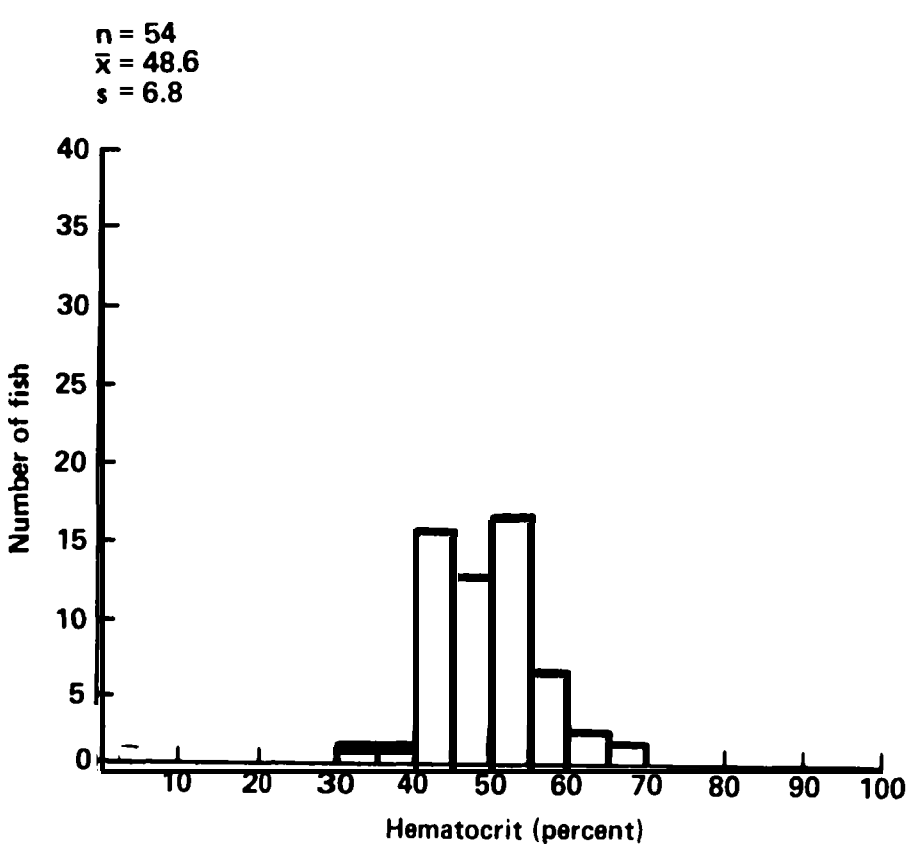
Wedemeyer (unpublished data) found mean hematocrit values of 40% (±3) and hemoglobins of 9 g/100 ml (+3> when he sampled Dworshak steelhead smolts in 1977. This is considerably lower than the values we obtained in 1978. The summarized data for the hematocrit and hemoglobin values for the Dworshak hatchery steelhead are presented in Figure 3. The mean hematocrits (52.4%) and hemoglobins (11.5 g/100 ml blood) are higher than the Tucannon Hatchery steelhead, and 78.0% of the hematocrit and 88.3% of

DISEASE LAB CODE 4601–4660

DATE SAMPLED May 17,1978

SPECIES STEELHEAD

HATCHERY STOCK TUCANNON



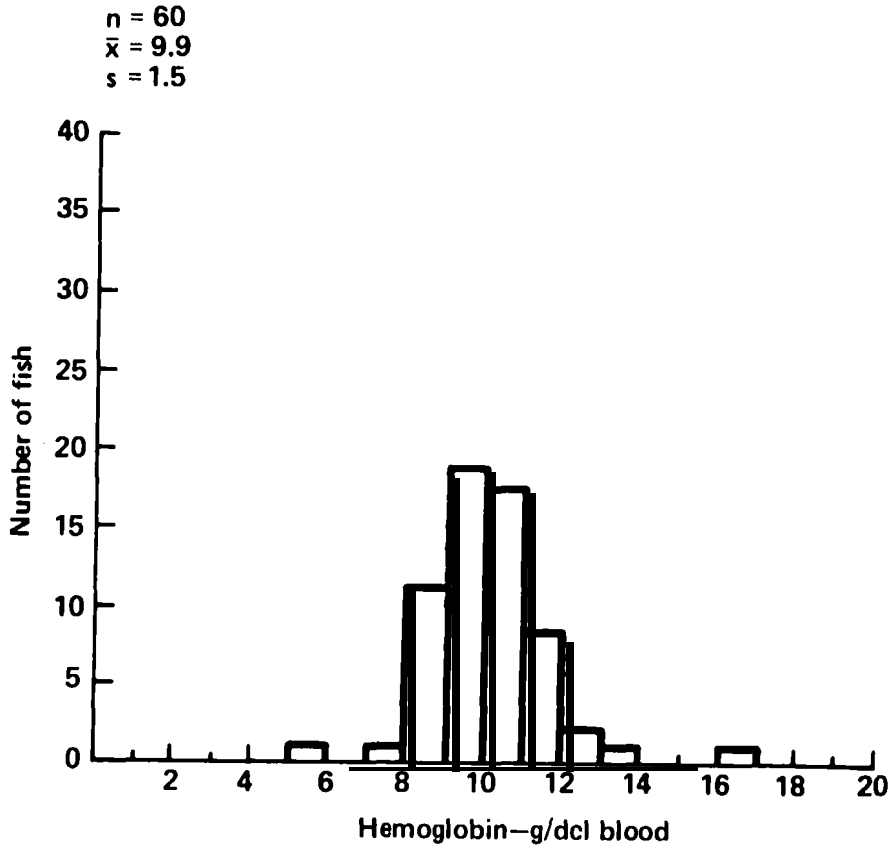


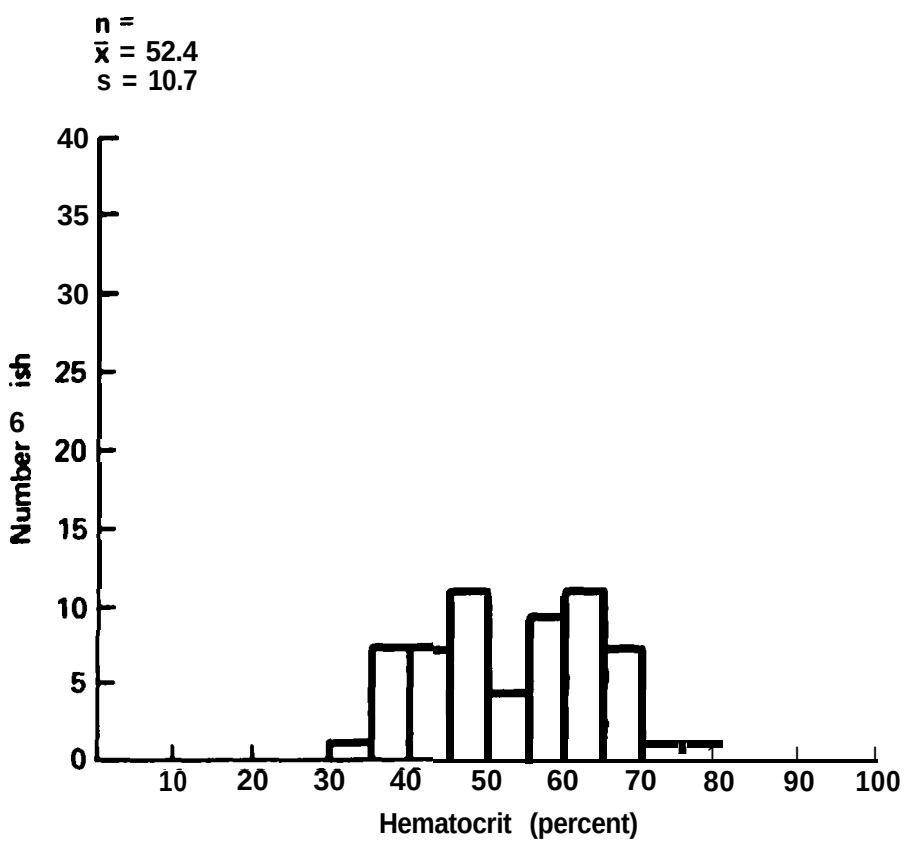
FIGURE 2.--A histogram and other data for tocrit and hemoglobin values of the Tucannon steelhead

DISEASE LAB CODE 4061–4120

DATE SAMPLED April 28,1978

SPECIES STEELHEAD

HATCHERY STOCK DWORSHAK



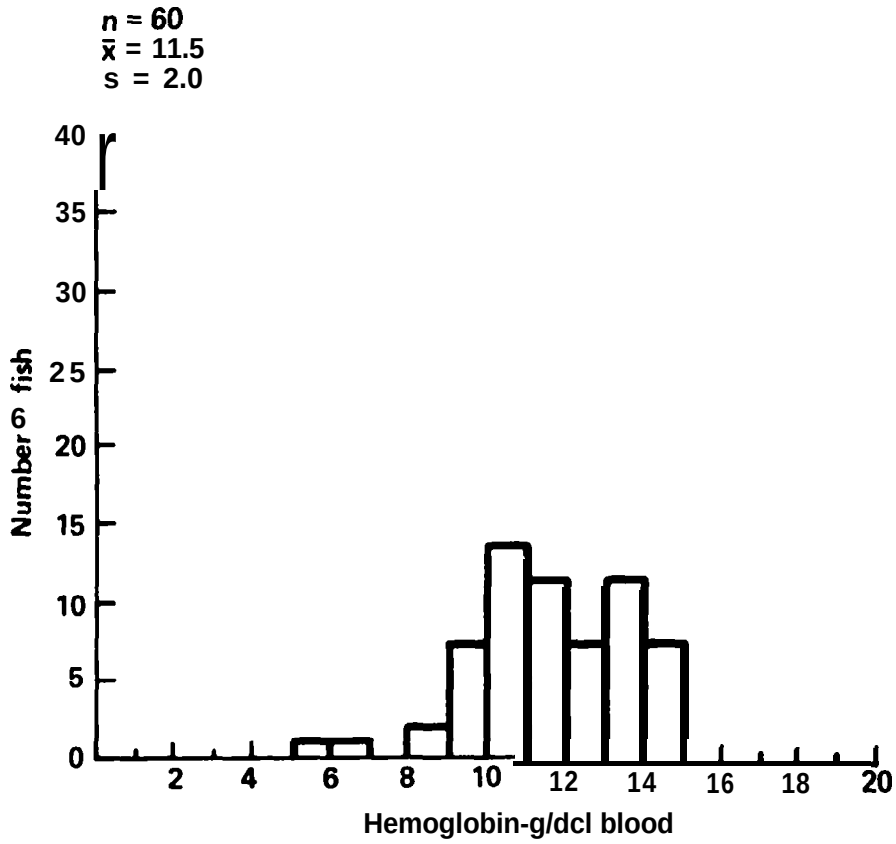


FIGURE 3.--A histogram and other data for hematocrit and hemoglobin values of the Dworshak steelhead.

the hemoglobin values were higher than the maximum expected values reported by Wedemeyer and Yasutake (1977) for clinically healthy rainbow trout. None were lower than the minimum. All blood samples for hematology and plasma electrolytes were collected at the Dworshak Hatchery. Therefore, these elevated values cannot be attributed to transportation stress.

3. CHELAN HATCHERY.

The summarized data for the henatocrit and hemoglobin values for the Chelan-Leavenworth Hatchery steelhead are presented in Figure 4. The mean values are within the ranges that Wedeneyer and Yasutake (1977) reported could be expected for rainbow trout, but 50.0% of the hematocrit values are above the expected maximum of 43%, and 31.7% of the hemoglobin values were above 9.3 g/100 nl blood. Sone of the hematocrit values were below the minimum expected (24%), and only 1.7% were below the minimum hemoglobin value.

4. WELLS-WINTHROP HATCHERY.

The summarized data for the hematocrit and hemoglobin values for the Wells-Winthrop Hatcheries steelhead are presented in Figure 5. The mean values were the highest of any of the steelhead groups studied, as 94.7% of the hematocrits were above the expected maximum (43%) for rainbow trout, and 85.0% of the hemoglobins were above the naximum value expected (9.3 g/100 ml).

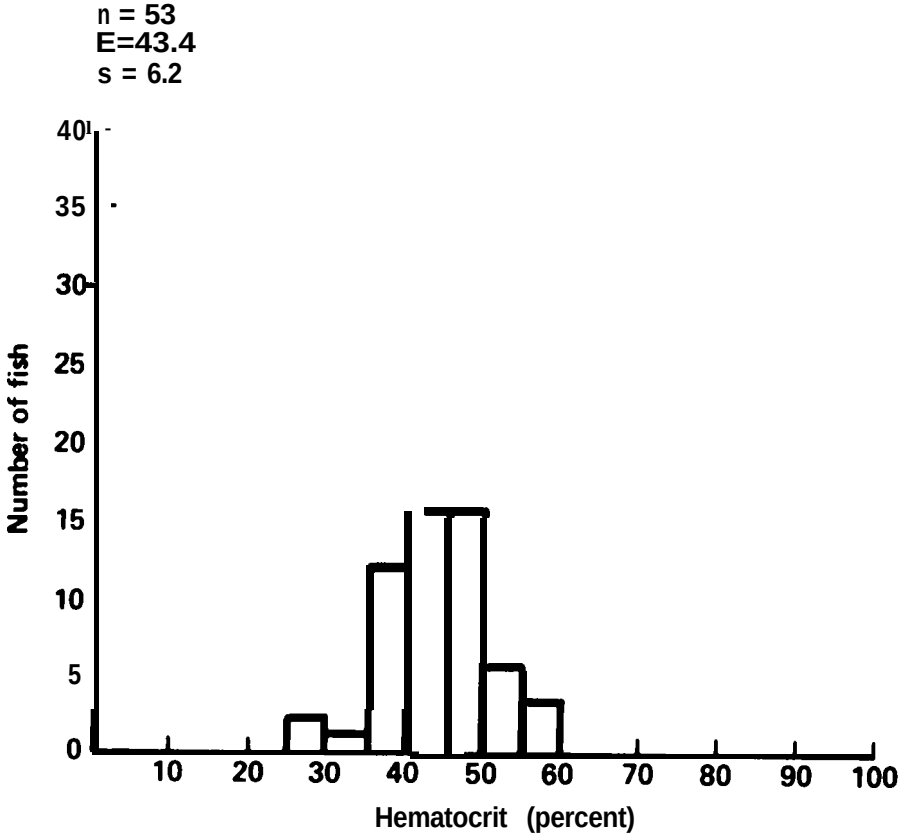
The generally high mean hematocrit and hemoglobin values for all of the steelhead stocks may reflect a normal hematological condition for these anadromous strains of the rainbow trout; or, they may be associated with mild dehydration as one physiological aspect of smoltification.

DISEASE LAB CODE 3901-3960

DATE SAMPLED May 4,1978

SPECIES STEELHEAD

HATCHERY STOCK LEAVENWORTH (Chelan)



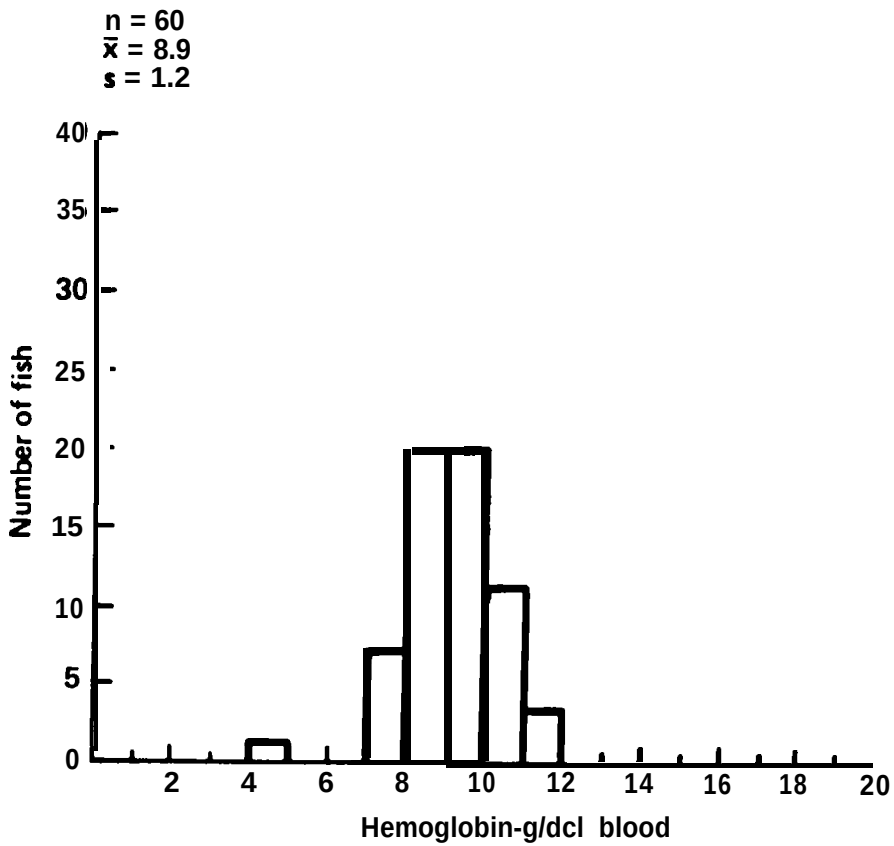


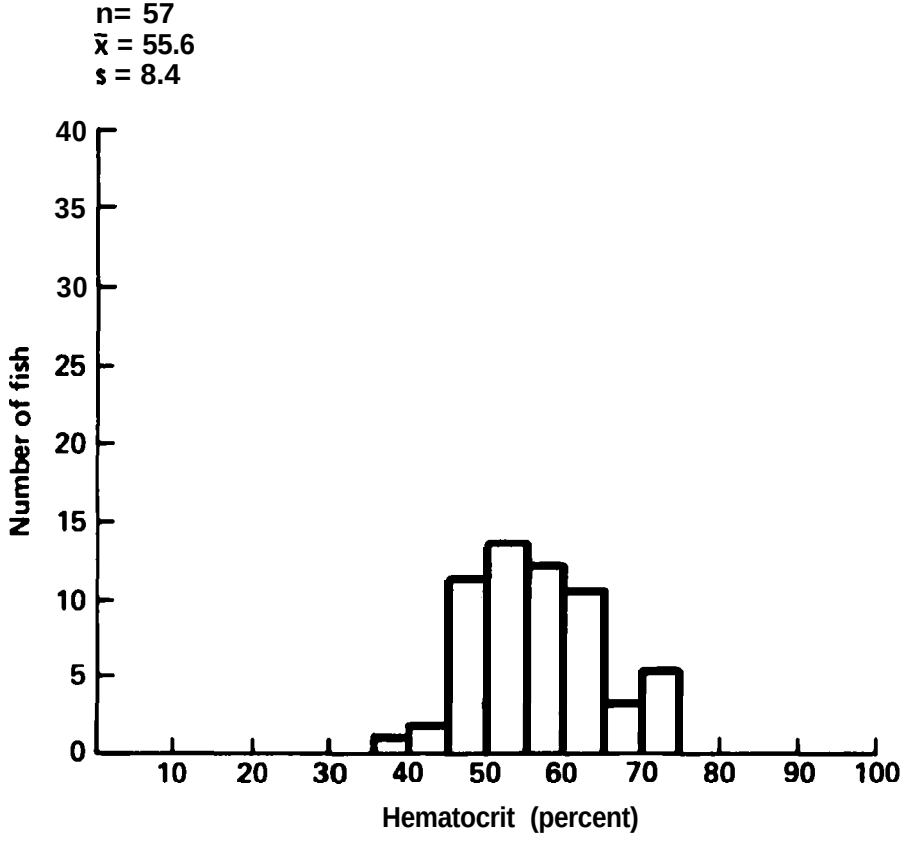
FIGURE 4.--A histogram and other data for the hematocrit and hemoglobin values of the Chelan-Leavenworth steelhead.

DISEASE LAB CODE 4501-4560

DATE SAMPLED May 9, 1978

SPECIES STEELHEAD

HATCHERY STOCK WINTROP (Wells)



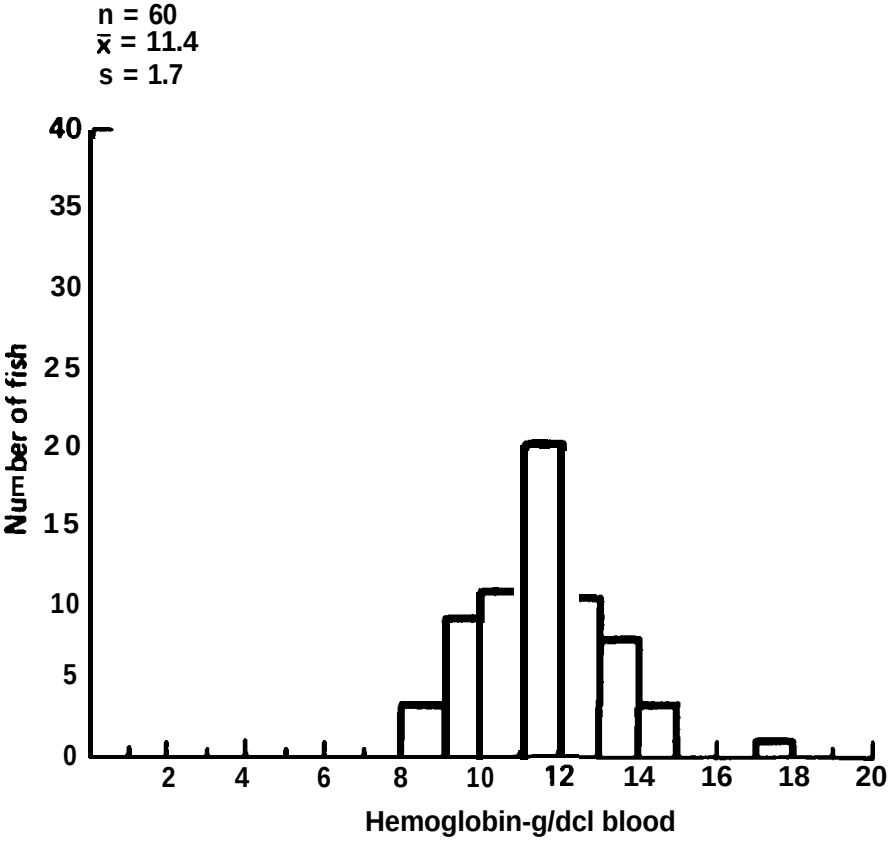


FIGURE 5..--A histogram and other data for the hematocrit and hemoglobin values of the Wells-Winthrop steelhead.

D. VIRAL SCREENING.

No confirmed evidence of common viral pathogens could be found by the private contract laboratory in the Tucannon or Chelan-Leavenworth Hatchery steelhead, but infectious pancreatic necrosis (IPN) virus was confirmed in the Dworshak and Wells-Winthrop populations. The complete results of the viral screening are presented in Appendix A.

E. INDIRECT FLOLJRESCENT ANTIBODY TEST FOR BACTERIAL KIDNEY DISEASE (IFAT-BKD), AND RELATIVE SURVIVAL OF STEELHEAD IN THE SEAWATER PENS.

1. TUCANNON HATCHERY.

The incidence of BKD as determined by IFAT was 13 out of 60 (21.6%), with 6 fish (10.0%) positive for both anterior and posterior kidney involvement; 5 (8.3%) with anterior kidney involvement only; and 2 (3.3%) with posterior kidney involvement only (Table 3).

The 30-day survival of the fish cultured in the seawater net-pens was 86%; the full term survival was 36% (which is relatively high for steelhead cultured in net-pens); and, no gross BKD lesions were found in necropsied fish (see Table 3).

2. DWORSHAK HATCHERY.

The incidence of BKD in this group as detected by IFAT was 16.7%. The level of infection was light, as only 1.7% of the fish had involvement in both posterior and anterior kidney; 10.0% in anterior kidney only; and 5.0% in the posterior kidney only (Table 3).

The 30-day survival of the Dworshak steelhead in the seawater net-pens was 46.42, and the survival through termination was approximately one-tenth that of the Tucannon Hatchery steelhead. None of the mortalities

oosted could be attributed to BKD, and only 26.6% of the nortalities posted could be attributed to bacterial pathogens (Table 3). A small percentage of the nortalities posted were due to ERM disease or furunculosis. Whether these were latent or transmitted in seawater is not known, as the hatchery records (Tahle 2) made availahle only indicate a treatment for external parasites with Formalin.

3. CHELAN-LEAVENWORTH HATCHERY.

The incidence of BKD in this group as detected by IFAT was 86.7% with 56.7X involvement in both anterior and posterior kidney (Table 3). The 30-day survival of the fish cultured in the seawater net-pens was 80.0%; the full term survival was 15.6%, and no BKD lesions were seen in the necropsied fish (see Table 3).

The hatchery records (Table 2) indicate that this stock suffered a 37.5% mortality during freshwater rearing and were fed medicated diets to treat a number of diseases.

4. WELLS-WINTHROP HATCHERY.

The incidence of BKD in this group as detected by IFAT was 85.0% with 46.7% involvement in both anterior and posterior kidney (Table 3). The 30-day survival of the fish cultured in the seawater net-pens was 10.5%, the full term survival was 2.2%, and no BKD lesions were seen in posted mortalities. The large mortality during the first period was attributed to osnoregulatory stress (Table 3).

No statistically significant correlations were found between the incidence OF BKD by TFAT and hematocrits or hemoglobins in the steelhead stocks studied.

HI STOPXTHOLOGY .

A detailed report on the examination and interpretation of selected tissue sections from the random samples is presented by the contract veterinary pathologist in Appendix B. A summary of the pathological conditions observed, their severity, and their frequency of occurrence is presented in Table 4. The severity is ranked as: I. Recognizable (least severe); II. Intermediate; and III. Severe . Sote that the incidence of rank 11 and III severity was low for all conditions encountered (Table 4).

Although the pathologist did not find gram-positive bacteria in either the kidney or liver tissue of the three steelhead stocks exanined, the more sensitive TFAT tests of kidney tissue smears from the same specimens did reveal the presence of BKD organisims.

In general, the pathological conditions observed in the three steelhead stocks were not wide-ranging and nay not significantly effect honing response or survival. However, there were several dominating conditions that appeared in all three stocks, and it may be of interest to sunnarize the probable causes.

Analysis of the pathologist's data indicate that all of the steelhead stocks had lesions (both degenerative and regenerative) in the skeletal muscle of the eye; both recognizable and of internediate severity.

Recognizable retrobular fat necrosis (death of fatty tissue behind the eyeball) was evident in the Dworshak steelhead. These ocular lesions may be the result of an unfulfilled nutritional requirement and/or possible nutritional disorder.

TABLE 4.--Pathological conditions observed in the homing stocks and their percentage of incidence.

Organ & pathology	Incidence (%)																							
	Tucannon steelhead				Dworshak steelhead				Wells-Winthrop steelhead				Kooskia spring chinook				Carson coho				Willard coho			
	Severity 1/				Severity 1/				Severity 1/				Severity 1/				Severity 1/				Severity 1/			
	I	II	III	Total	I	II	III	Total	I	II	III	Total	I	II	III	Total	I	II	III	Total	I	II	III	Total
Eye																								
Skeletal muscle lesions	40.3	8.3	0	56.7	51.3	10.0	0	61.3	50.0	0	1.7	51.7	10.0	0	0	10.0	27.1	0	0	27.1	36.8	3.5	0	40.3
Retrobulbar fat necrosis	0	0	0	0	27.1	3.3	1.7	32.1	6.7	0	0	6.7	6.7	1.7	0	8.4	0	0	0	0	6.9	0	0	6.9
Minimal subacute iridocyclitis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	0	1.7	0	0	0	0
Choroid gland inflamation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	0	1.7	0	0	0	0
Focal mononuclear cells in optic nerve	0	0	0	0	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chronic active ophthalmitis	0	0	0	0	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corneal scleritis	0	0	0	0	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Optic nerve neuritis	0	0	0	0	3.3	0	0	3.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Segmental vasculitis	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chronic conjunctivitus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	0	1.7
Gills																								
Increased number of lymphocytes	23.3	0	0	23.3	63.3	10.0	3.3	76.6	65.0	1.7	0	66.7	73.3	20.0	1.7	95.0	74.6	0	0	74.6	22.4	0	0	22.4
Epithelial cell proliferation	31.7	0	0	31.7	66.7	16.7	1.7	85.1	26.7	11.7	0	88.4	10.0	6.7	0	16.7	61.1	0	0	61.1	41.4	3.5	0	44.9
Basophillic granular organism	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	60.7	16.1	0	76.8	5.2	3.5	1.3	10.4
Filamentous bacteria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	0	1.7	0	0	0	0
Ciliated protozoan parasites	0	0	0	0	23.3	0	0	23.3	0	0	0	0	3.3	0	0	3.3	0	0	0	0	0	0	0	0
Possible protozoan parasites	0	0	0	0	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sporozoan parasites	0	0	0	0	3.3	0	0	3.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Local capillary dilation of the secondary lamellae	1.7	0	0	1.7	0	0	0	0	8.3	0	0	8.3	0	0	0	0	0	0	0	0	0	0	0	0
Microsporidian-sporozoan parasites	0	0	0	0	0	0	0	0	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0
Neutrophils in secondary lamellae	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Congestion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	0	1.7
LIVER																								
Increased parenchymal fat	8.3	0	0	8.3	11.7	0	0	11.7	3.3	0	0	3.3	3.3	0	0	3.3	1.7	0	0	1.7	0	0	0	0
Bacterial kidney disease with gram pos. bacteria	0	0	0	0	0	0	0	0	0	0	0	0	3.3	1.7	0	5.0	0	1.7	0	1.7	0	0	0	0
Granulomatous lesions w/o gram pos. bacteria	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	1.7	1.7	3.4	0	0	3.4	1.8	0	0	1.8
Focal mononuclear cell infiltrate	20.0	0	0	20.0	0	0	0	0	0	0	0	0	48.3	15.0	5.0	68.3	5.1	0	0	5.1	7.0	0	0	7.0
Focal necrosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	0	1.7
Gram stain	0	0	0	0	0	0	0	0	0	0	0	0	3.3	0	0	3.3	1.7	0	0	1.7	0	0	0	0
KIDNEY																								
Bacterial kidney disease with gram pos. bacteria	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	3.3	5.0	0	1.7	0	1.7	0	0	0	0
Granulomatous lesions w/o gram pos. bacteria	6.7	0	0	6.7	1.7	0	0	1.7	1.7	0	0	1.7	23.3	3.3	0	26.6	1.7	1.7	0	3.4	1.7	1.7	0	3.4
Nephrocalcinosis	0	0	0	0	0	0	0	0	0	0	0	0	23.3	8.0	3.3	34.6	0	0	0	0	0	0	0	0
Gram pos. debris in tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.8	0	0	6.8	0	0	0	0
Debris in excretory duct	0	0	0	0	0	0	0	0	1.7	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0
Gram stain	0	0	0	0	0	0	0	0	0	0	0	0	3.3	0	0	3.3	1.4	0	0	1.4	0	0	0	0

1/ I = Recognizable (least severe)
II = Intermediate
III = Severe

The pathological conditions in gill tissue were dominated by increased numbers of lymphocytes in gill tissue and epithelial cell proliferation. These observations may be indicative of very mild exposure to antigens, including pathogenic micro-organisms, and the mildest possible form of nutritional gill disease. Second in frequency of occurrence was the presence of ciliated protozoan parasites in the Dworshak steelhead. The incidences of gill pathology were the least frequent in the Tucannon steelhead.

The available hatchery records (Table 2) indicate that the steelhead mortality in the Tucannon Hatchery was 43% and in the Wells-Winthrop pond and hatchery, 28%.

II. SPRING CHINOOK SALMON: KOOSKIA HATCHERY (PATHOLOGY SAMPLE NUMBERS 4001-4060).

A. GILL ENZYME ANALYSES.

Spring chinook salmon from Kooskia Hatchery had an average gill Na+-K+ ATPase activity of 18.1 ± 2.8 on 24 April. Values ranged from 14.0 to 22.7 (Table 3), and the activity continued to increase after transfer to seawater. A small group of O-age spring chinook salmon sampled at the same time had an average activity of 6.2.

B. PLASMA ELECTROLYTES.

Only plasma Na and Cl were determined for the Kooskia Hatchery spring chinook salmon (Table 3). The mean values (Na = 114 meq/l; Cl = 104 meq/l) were apparently lower than a Leavenworth Hatchery spring chinook salmon stock (Na = 150 meq/l; Cl = 108 meq/l), and the Kalama Falls Hatchery spring chinook salmon stock (Ka = 137 meq/l; Cl = 116 neq/l) sampled during the same period. There are apparently very little published data on plasma electolytes of spring chinook salmon.

C. HEMATOLOGY.

The summarized data for the hematocrit and hemoglobin values are presented in Figure 6. The mean values (39.1%; 6.6 g/dcl blood) are probably normal. Unpublished data from salmon diet studies in Oregon indicate expected mean hematocrits for spring chinook salmon ranging from 24.2 to 38.0% and 35 to 39% for fall chinook salmon. Our own experience has indicated that hematocrit values below 28% in Pacific salmon may be the beginning stages of a number of problems, and only 8.3% of the Kooskia Hatchery fish fell below this level.

D . VIRAL SCREENING (SEE APPENDIX A).

Rangen Laboratories found only 5 out of 12 pools negative for virus on the first screening, and IPN virus was confirmed in this stock.

E. IFAT-BKD.

The Kooskia Hatchery spring chinook salmon had the highest incidence of BKD (as determined by IFAT) for any of the homing stocks screened,with a total incidence of 90%, and a 70% incidence of infection in both kidneys (Table 2). It was not possible to determine if there was any correlation of BKD to hematology because of the low number of noninfected fish in the sample for comparative analysis.

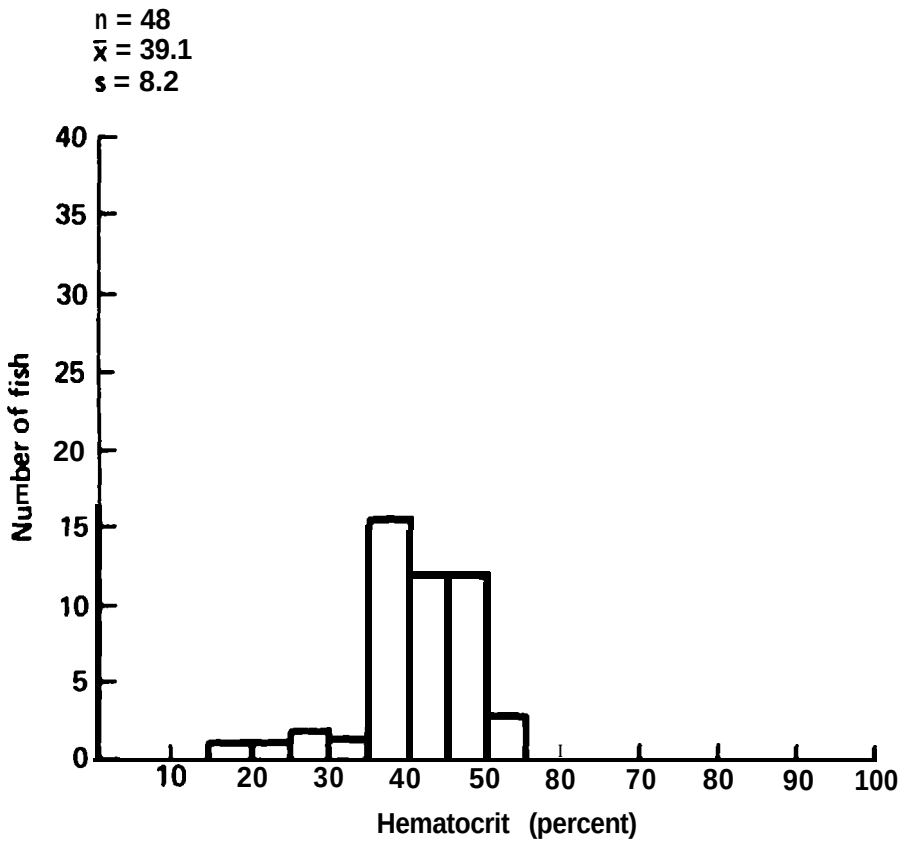
The 30-day survival of this group (Table 3) in the seawater net-pens was the highest of any homing stock tested in 1978 (98.8%). The seawater survival through termination of the tests (October 1978) was 36.7%, and 10.5% of the mortalities posted had grossly visible BKD lesions in the kidney, spleen, or liver. This follows the typical pattern that we

DISEASE LAB CODE 4001-4060

DATE SAMPLED April 25, 1978

SPECIES SPRING CHINOOK

HATCHERY STOCK KOOSKIA



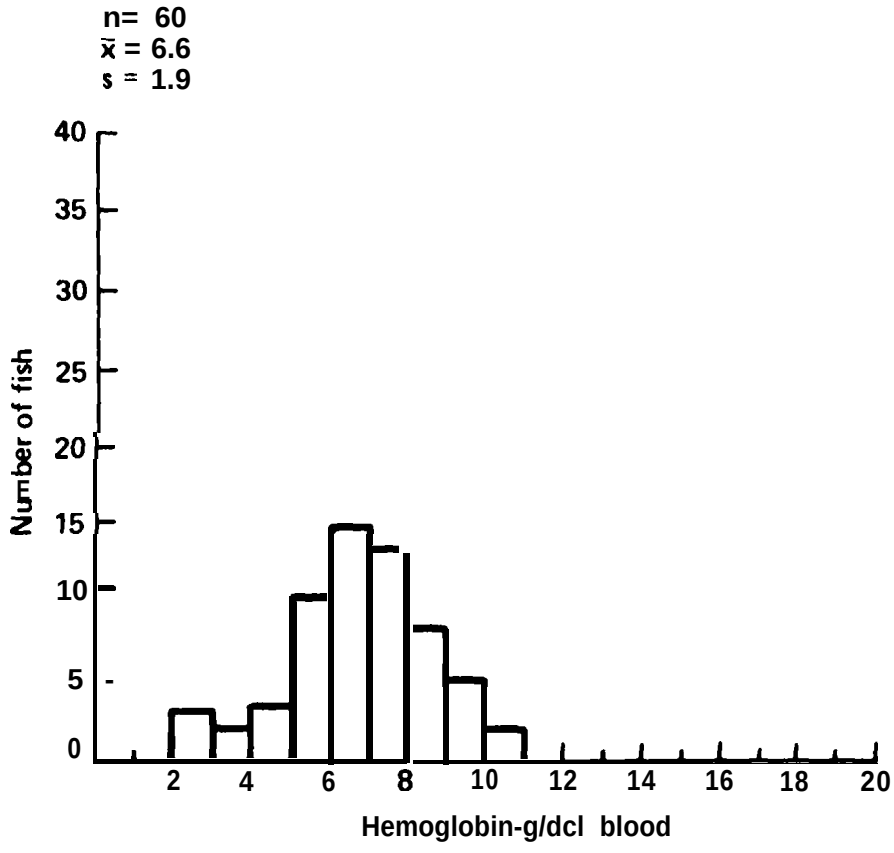


FIGURE 6.--A histogram and other data for hematocrit and hemoglobin volues for the kokia spring chinook salmon.

have seen in the past: mortality in seawater due to latent BKD infections generally begins in the fall and may take more than 2 years (Ellis et al. 1978). A small percentage of the mortalities posted from this group (Table 3) were from ERM disease or furunculosis. Whether this was latent or transmitted in seawater is not known.

F. HISTOPATHOLOGY (SEE APPENDIX B).

The major difference between the other homing stocks and the Kooskia Hatchery spring chinook salmon (Table 4) are: 1) the incidence of muscle lesions of the eye is l/5-1/6 that of the steelhead and l/3-1/4 that of the coho salmon; 2) there is a very high incidence (95%) of increased lymphocytic activity in the gills; 3) the pathologist was able to find BKD (by tissue gram stain) in both liver and kidney (5%), and even though the sensitivity was approximately l/20 that of the IFAT, the level of infection must have been comparatively greater than that in the steelhead; 4) granulomatous lesions (26.6%) and nephrocalcinosis (34.6%), a condition of calcium phosphate precipitation in the renal tubules, dominated any pathology of the kidney. If the latter conditions are secondary results of low-level BKD infections, they could impair the process of excretion.

The hatchery records (Table 2) indicate that this group was treated for external parasites with Formalin, which may account for some of the increased lymphocytic activity in the gill tissue.

III. COHO SALMON.

A. GILL ENZYME ANALYSES.

1. CARSON HATCHERY (PATHOLOGY SAMPLE NUMBERS 4401-4460).

The Carson Hatchery coho salmon were sampled on 27 April. Gill Na+-K+ ATPase activities averaged 11.1 + 1.3, ranging from 8.8 to 12.4.

Sone parr marks were still visible, but ventral fins were becoming clear. These indications suggested that smolt transformation had begun.

2. WILLARD HATCHERY (PATHOLOGY NUMBERS 4661-4700 AND 4351-4370).

A complete freshwater gill Xa+-K+ ATPase profile was conducted on the Willard Hatchery coho salmon.

The first external signs of smolting were noticed on 26 April, at a time when gill Na+-K+ ATPase activity was 10.2. Three releases were made to coincide with groups used in the homing study. A high percentage of the fish were at or near smolt stage on the first two releases (Figure 7), one coming before peak activity and the other (24 May) coming just as the Na+-K+ ATPase decline started. At the time of the third release (8 June) parr reversion was well in progress as indicated by the average Na+-K+ ATPase activity (about 9). Very few fish from these three releases were obtained from the Jones Beach recapture operation. Only one, from the third release, was obtained for Na+-K+ ATPase determination.

Average sizes of fish sampled near the three release times were: 1) 12.5 cm, 21.8 g; 2) 12.5 cm, 22.4 g; 3) 13.6 cm, 27.9 g.

B. PLASMA ELECTROLYTES.

Miles and Smith (1968) reported on plasma electrolytes in hatchery coho salmon. Spring samples of fish in fresh water averaging 12.5 g were as follows:

	X (meq/l)	Range (meq/l>
Na+	146.7	130.0-168.0
Cl-	117.3	90.9-132.6
K+	8.1	1.8 = 19.0

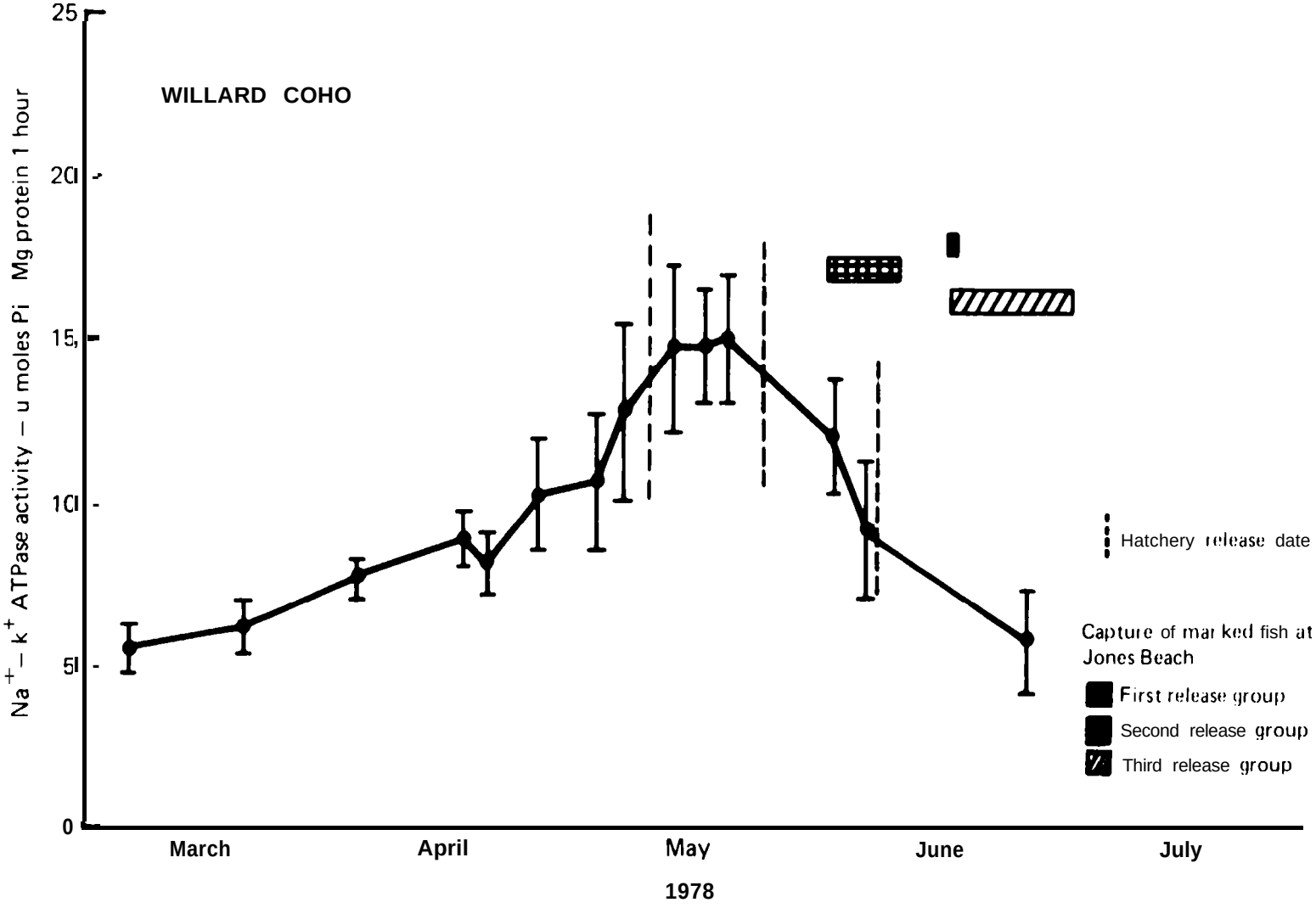


FIGURE Y.--Gill Na+ -K+ ATPase enzyme activity in Willard coho salmon sampled at the hatchery. Enzyme activity was measured at approximately 2-week intervals from early March through late June The data shown for each sampling period are the mean and variance of 10 analyses from 30 fish (pooled--3fish/analysis).

Our own studies of Columbia River Hatchery coho salmon in 1978 resulted in the following ranges of mean values for plasma electrolytes:

Na+ 140.8 - 170.0

cl- 113.4 - 140.8

K+ 5.7 - 8.4

1. CARSON HATCHERY.

The mean plasma electrolytes for the Carson Hatchery coho salmon (Table 3) were well within these ranges. The Na+ values ranged from 126 to 178 meq/l (x= 159.4). Cl- values ranged from 102 to 137 meq/l (x = 124.4), and K+ values ranged from 0.8 to 7.8 meq/l (X = 4.1).

2. WILLARD HATCHERY.

The mean Na+ (144.3 meq/1), Cl- (115.4 meq/l), and K+ (5.8 meq/l) values were well within the expected levels for clinically healthy coho salmon as prescribed by Miles and Smith (1968).

C. HEMATOLOGY.

Wedemeyer and Chatterton (1971) list normal expected values (for coho salmon) in fresh water of 32.5 to 52.5% for hematocrits and 6.5 to 9.9 g/100 ml blood for hemoglobins.

1. CARSON HATCHERY.

The mean hematocrits (32.9%) of the Carson coho salmon were close to the expected low, and the mean hemoglobin values (4.7 g/100 mg blood) were below the lowest expected value for clinically healthy fish (Figure 8). There were 41.3% of the hematocrit values below the expected low value (32.5%), and none of the fish were above the high (52.5%). About 91.4% of the hemoglobin values were below the expected minimum for clinically healthy coho salmon (6.5 g/100 mg), and none were above the high (9.9 g/100 ml).

DISEASE LAB CODE 4401-4460 4401-4460

DATE SAMPLED April 26, 1978

SPECIES COHO

HATCHERY STOCK CARSON

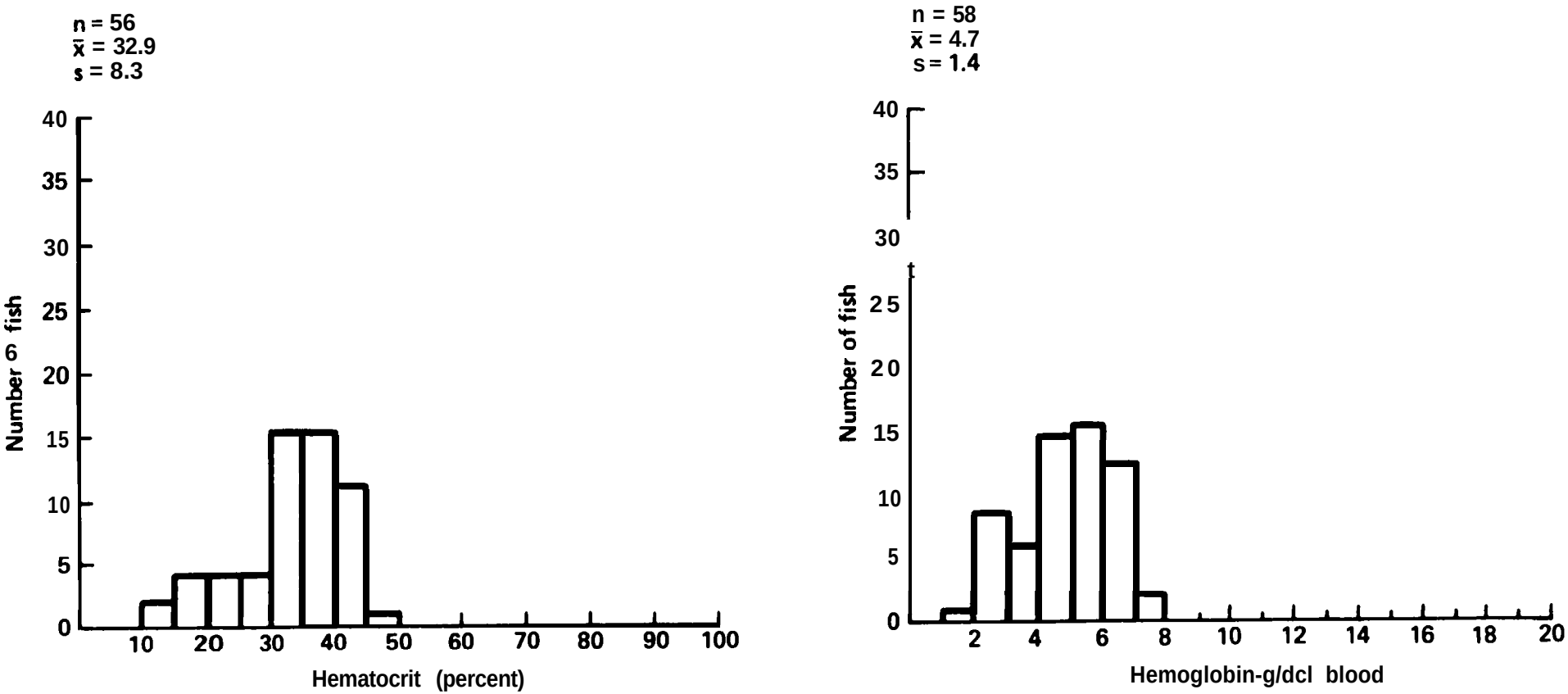


FIGURE 8.--A histogram and other data for hematocrit and hemoglobin values for the Carson coho salmon.

2. WILLARD HATCHERY.

The summarized data for the hematocrit and hemoglobin values are presented in Figures 9a, b, and c. Mean hematocrits from all three release groups (Table 3) were in the middle of the range expected for clinically healthy coho salmon (32.5 to 52.5%) as proposed by Wedemeyer and Chatterton (1971). The mean hemoglobin values (6.4 g/100 ML blood) of the first release group (I) were slightly below the expected (6.5 to 9.9 g/100 ml). The hematocrit values (Figure 9a) of the Willard I coho salmon were widely spread.

The change in the percentage of fish above or below the expected values of hematocrit and hemoglobin are listed below. Miles and Smith (1968) reported a decrease in hematocrits in coho salmon yearlings in early April followed by an increase in early May, but they did not report on hemoglobin values. In the case of Willard Hatchery coho salmon, there is a continuing shift of both mean and expected values to a higher level with the progression of spring. This could be a physiological adjustment to minor decreases in available oxygen.

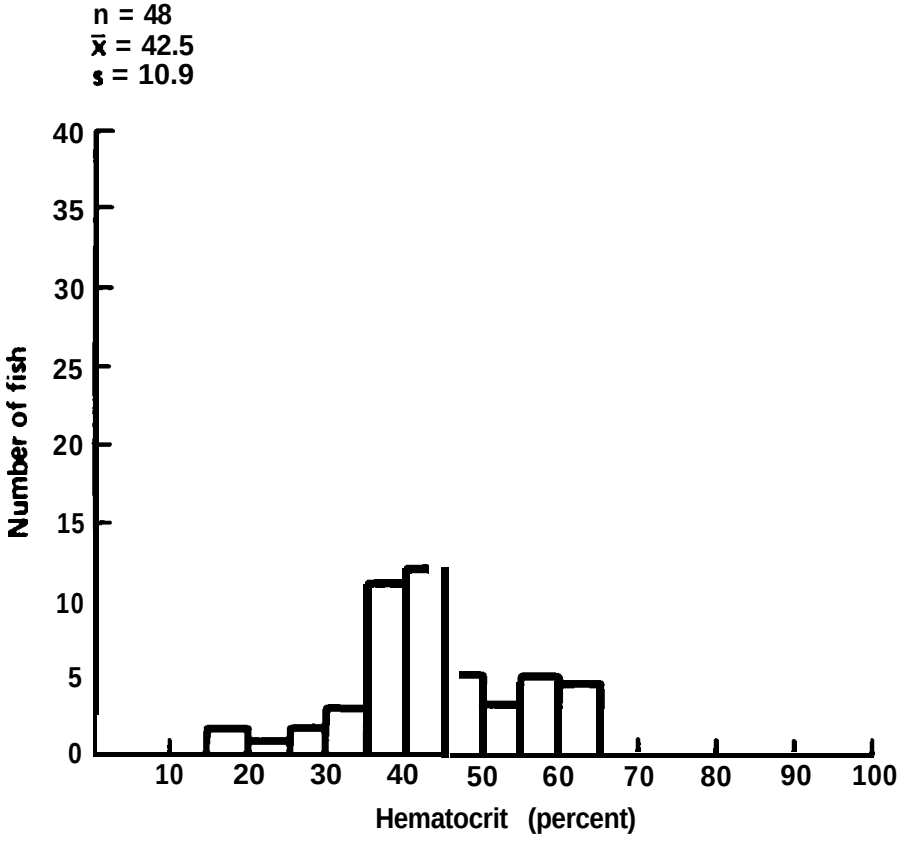
	Hematocrits		Hemoglobins	
	% of normal expected values		% of normal expected values	
Release group	Below (%)	Above (%)	Below (%)	Above (%)
I	10.2	18.4	56.3	0
II	5.7	9.4	29.3	3.4
III	6.9	3.4	5.1	13.5

DISEASE LAB CODE K01–K60

DATE SAMPLED May 15, 1978

SPECIES COHO

HATCHERY STOCK WILLARD I



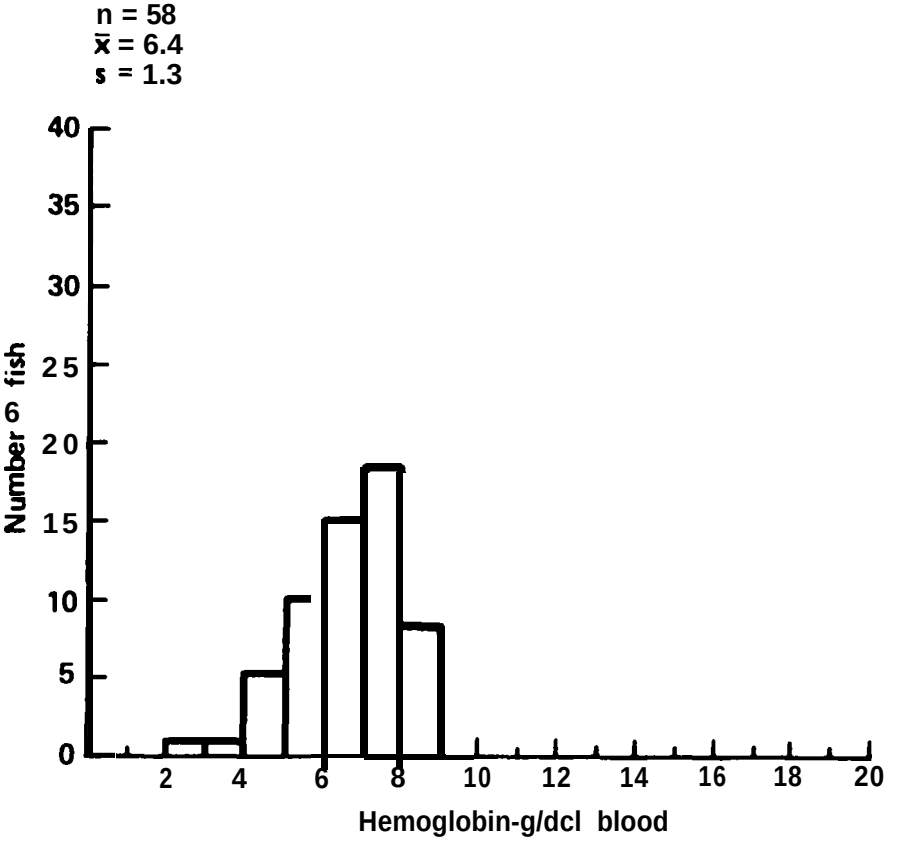


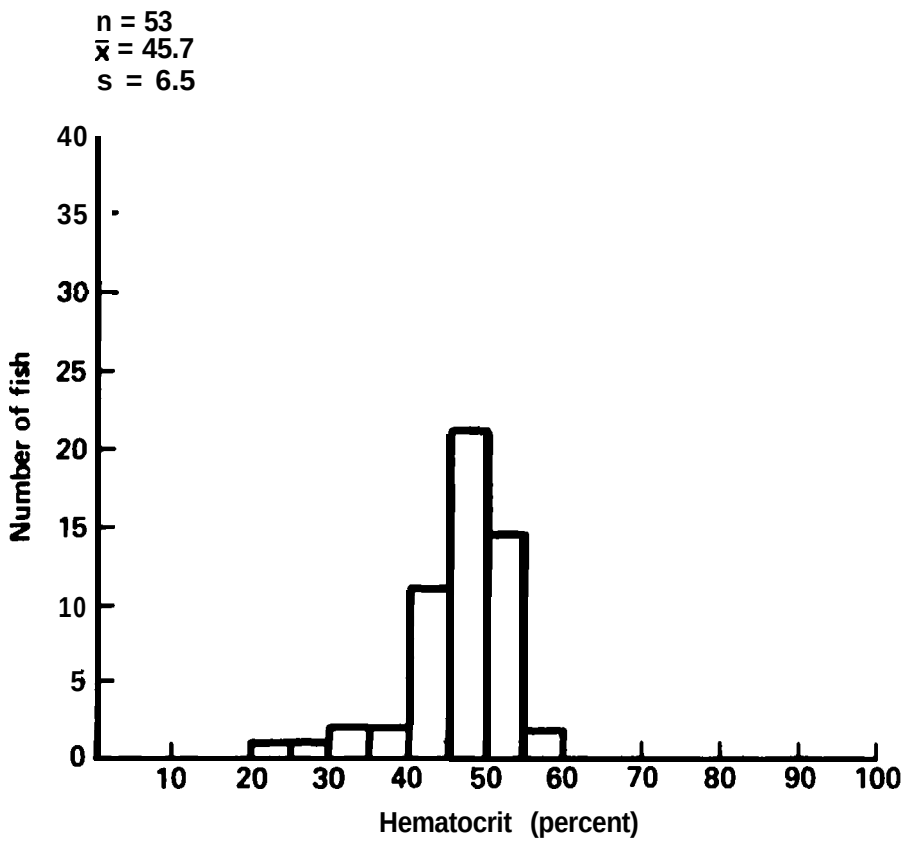
FIGURE 9A.--A histogram and other data for hematocrit and hemoglobin values for the Willard (I) coho salmon-first release group.

DISEASE LAB CODE N01–N60

DATE SAMPLED May 25,1978

SPECIES COHO

HATCHERY STOCK WILLARD II



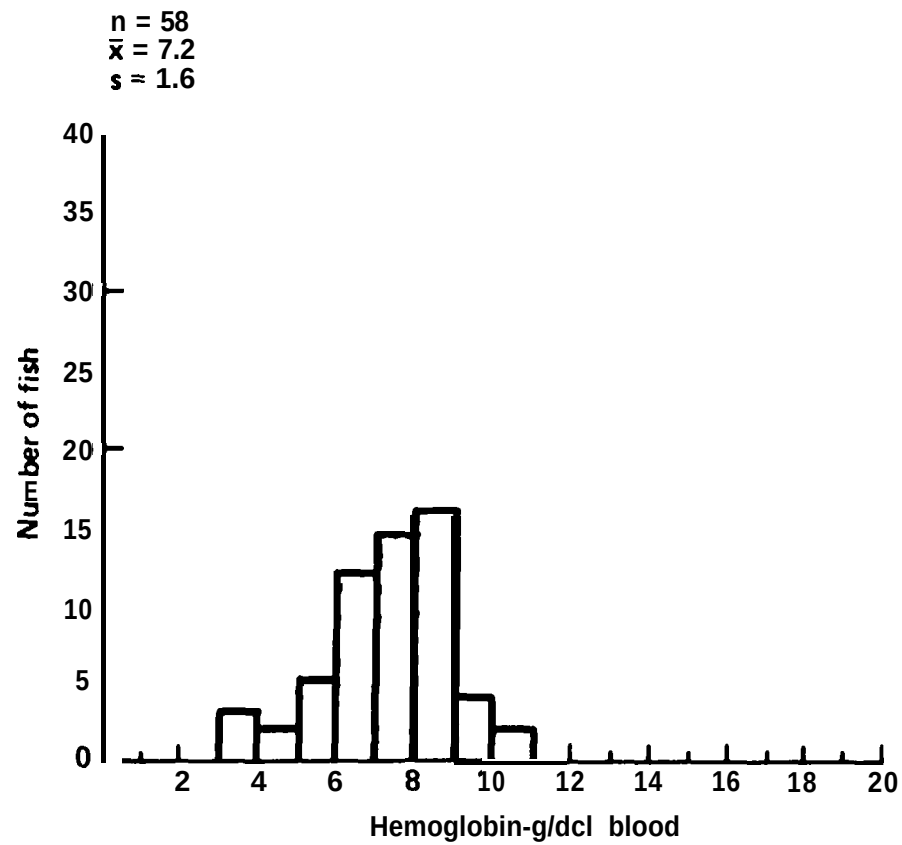


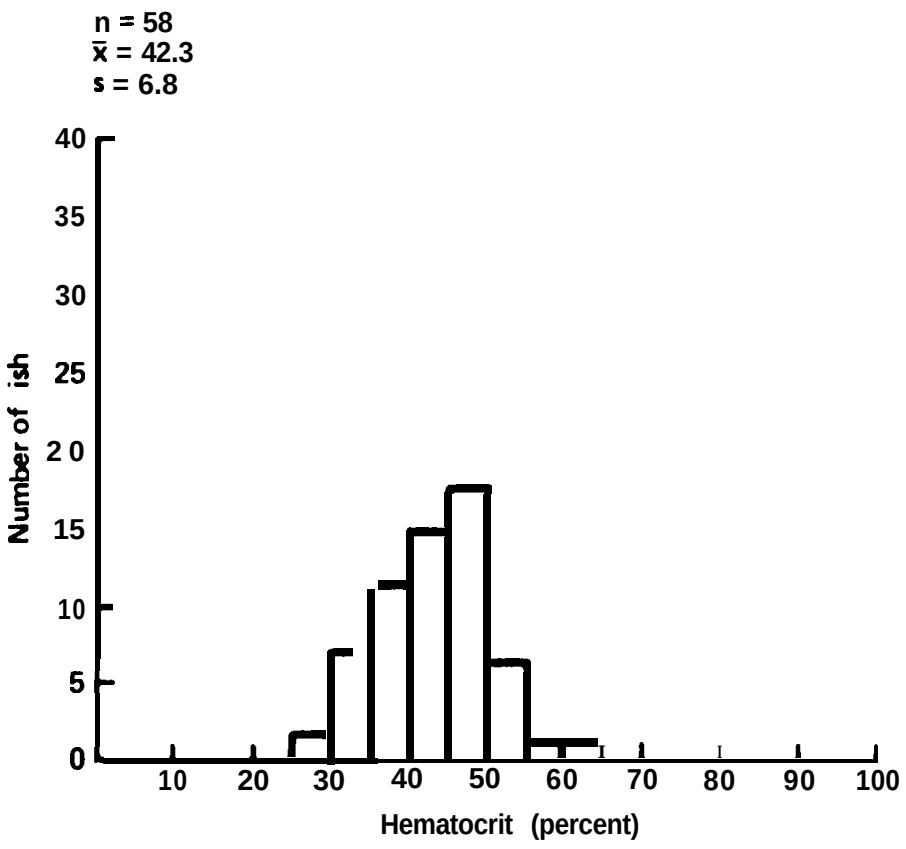
FIGURE 9b.--A histogram and other data for hematocrit and hemoglobin values for the Willard (II) coho salmon-second release group.

DISEASE LAB CODE 435 I-4370 4661-4700

DATE SAMPLED June 12,1978

SPECIES COHO

HATCHERY STOCK WILLARD III



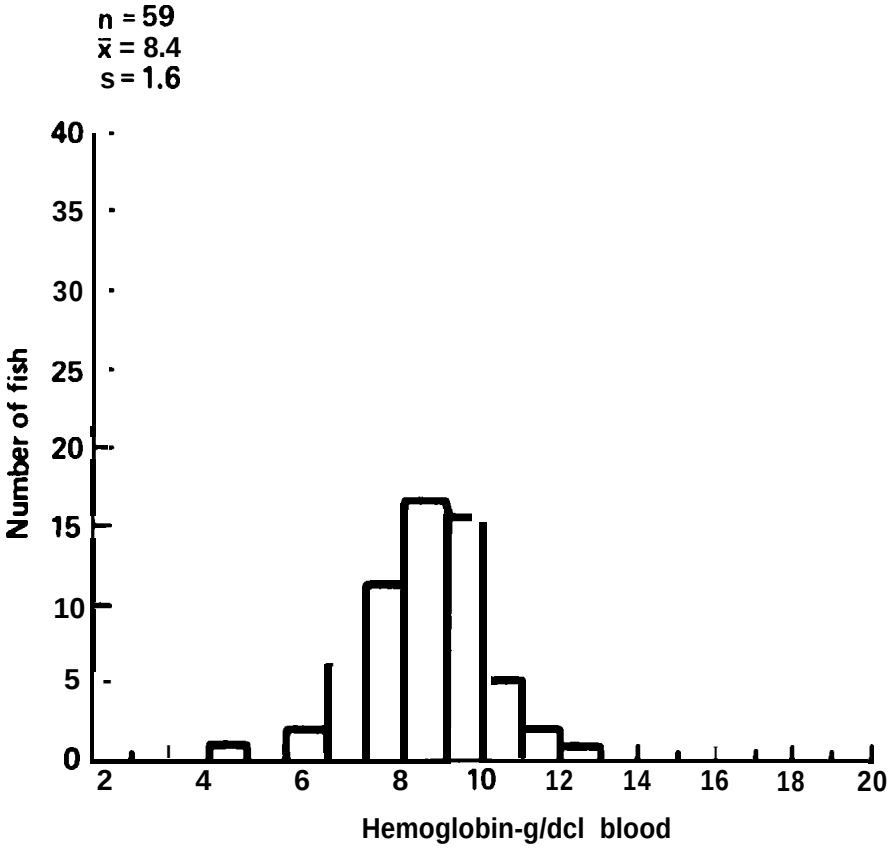


FIGURE 9c .--A histogram and other data for hematocrit and hemoglobin values for the Willard (III) coho salmon-third release group.

D. VRIAL SCREENING (SEE APPENDIX A).

1. CARSON HATCHERY.

The Rangen Laboratories only found 6 out of 12 pools to be negative for virus in this stock, and IPN virus was confirmed.

2. WILLARD HATCHERY.

Rangen Laboratories conducted tests on the Willard Hatchery II and III release groups only. In the II group, there were no negative pooled samples, and IPN viruses could not be confirmed. In the III group, there were five negative pooled samples, and IPS virus was confirmed in some of the remaining positive groups.

E. IFAT-BKD.

1. CARSON HATCHERY.

The incidence of BKD as determined by IFAT was only 5%, and there were no demonstrably abnormal hematocrit or hemoglobin values in the infected fish.

The 30-day survival for the Carson coho salmon was 98.2%, and the full-term survival (46.1%) was the highest of any of the 1978 homing stocks exanined (Table 3). In spite of the low incidence of BKD, 4.8% of the mortalities exanined were determined to have gross BKD lesions in the kidneys.

2. WILLARD HATCHERY.

Kidneys were sampled from all three release groups for BKD screening. The incidence of infection between group I and II was almost identical (Table 2), but 2 weeks later (group III), the percentage of BKD infected fish rose sharply. Most of this increase was in what might be

considered new hosts (anterior or posterior only). Because of the small sample size, it may not be possible to relate the incidence of BKD to hematocrit or hemoglobin levels. However, the data (below) do show a trend of decreasing hematocrit values with increasing latent BKD infection in the kidney.

	% Hematrocrit			g hemoglobin/100 ml blood		
	n	Mean	Variance	n	Mean	Variance
No detectable BKD	34	43.8	7.1	35	8.9	1.4
Anterior or post-erior BKD only	18	41.3	5.2	18	7.8	1.2
Both posterior and anterior kidney infected	5	37.6	6.8	5	8.2	1.2

There were mortalities from gross BKD in all three groups cultured in the seawater net-pens (Table 3), and the percentage of mortalities with grossly detectable BKD was highest for the group III fish. The initial (30 day) survival in all 3 groups was quite high (and similar), but the survival to termination of the tests was only 34 to 41%. This high early survival in all 3 groups at least indicates that osmoregulatory stress was minimal. The 7.9% mortality in the group III fish due to gross BKD is disturbing in that it indicates a possible delayed mortality due to latent BKD in this stock.

F. HISTOPATHOLOGY (SEE APPENDIX B).

1. CARSON HATCHERY.

There was a recognizable incidence of skeletal muscle lesions of the eye (27.1%), recognizable increased numbers of lymphocytes (74.6%) and epithelial cell proliferation (61.0%) in the gills, and a 76.8% incidence of basophillic granular organisms in the gills (Table 4). These latter structures stained gram negative and may represent microsporidian protozoan parasites.

The incidence of BKD as detected by tissue gram stain was 1.7% in the liver and 1.7% in the kidney. Even though the incidence of BKD as detected by IFAT and tissue gram strain was low, tissue involvement in individually infected fish in the total population must have been significant because we would not expect a 4.8% mortality in the sea-pens from BKD with such a low Incidence level (5% by IFAT). There was gram-positive debris in the kidney tubules (6.8%), and other gram staining material in the tissue (3.4%).

Table 2 is the hatchery record for the Carson Hatchery coho salmon, and indicates the fish were treated for furunculosis and probably some external parasites, but not BKD.

In general, these fish were in excellent condition, but the total evidence suggests that there could be a long term mortality due to latent BKD of at least 5%.

2. WILLARD HATCHERY (GROUP III FISH ONLY).

Recognizable pathological conditions of the eye were at similar levels to those of the Carson Hatchery coho salmon, but there was a considerable reduction in the percentage of fish with any gill pathology,

particularly those fish with evidence of basophillic granular organisms (Table 4). There were no detectable incldences of BKD organisms in liver or kidney (as determined by tissue gram stain), no evidence of gram positive staining debris, and no evidence of other gram stained material in tissue. This would seem to indicate that the severity of individual BKD infections was less than that of the Carson Hatchery coho salmon. However, the fact that the known BKD mortalities in seawater culture ranged from 2.9 to 7.9%. and that the IFAT-BKD levels ranged from 15.0 to 40.7% should be sufficient evidence to indicate that latent BKD could be a serious problem and might be the direct cause of a 5 to 10% mortality in the ocean.

Table 2 lists the available hatchery records of the Willard Hatchery coho salmon. Although BKD and furunculosls were two of the diseases detected in these fish, there was no evidence of furunculosis in seawater mortalities posted. This may have been the result of effective medication (for gram negative organisms) with TM-50.

COMPARATIVE VIRAL ASSAYS FROM SURVIVING FISH IN SEAWATER NET-PENS

Three hundred fish from each of the stocks of fish sampled during the course of this study had been maintained in salt water at the NMFS Manchester Laboratory since the time of sampling as part of the test for saltwater adaptation and survivability. In an attempt to expand upon the results of the viral Isolation tests during the course of the freshwater smolt phase of the study, those stocks surviving saltwater introduction in sufficient numbers were sampled by USFWS personnel from the National Fisheries Research Center in Seattle, Washington, according to the methods described previously in this report. Paired tissue pool samples were tested for the presence of virus by both the National Fisheries Research Center and the Rangen Research Laboratory.

A total of 11 saltwater stocks and one additional stock that had been held in fresh water at the NMFS Yontlake Laboratory in Seattle were examined. All twelve stocks had been found to be infected with IPN virus at the time of hatchery release as reported in the foregoing report but were found (by both USFWS and Rangen) to be negative for virus after a saltwater maintenance period of 155 to 200 days. Based upon the evidence for variation in the clinical presence of IPN virus and infectious henatopoetic necrosis (IHS) with regard to age, species, and stress conditions, it is not unreasonable to assume that the virus could well have returned to subclinical undetectable levels following a reduction in smoltification stress by acclimation to salt water. Serological surveillance of these same stocks may have provided a tool with which prior exposure could have been demonstrated in the absence of any clinical isolations of the virus.

SUMMARY AND CONCLUSIONS

The health status of the stocks was quite variable as could be expected. The Dworshak and Wells Hatcheries steelhead suffered from some early stresses in seawater, probably osmoregulatory. The incidences of latent BKD in the Wells and Chelan Hatcheries steelhead and Kooskia Hatchery spring chinook salmon were extremely high, and how these will effect survival in the ocean is not known. Gill enzyme activity in the Dworshak and Chelan Hatcheries steelhead at release was low. Of the s teelhead, survival in the Tucannon Hatchery stock will probably be the highest, with Dworshak Hatchery stock the lowest.

The analyses conducted by the veterinary pathologist indicate that overall there was no evidence of serious pathological conditions that might be disastrous to any given stock, but at this time it is also difficult to interpret the results of certain types of clinical pathology that have either not been previously reported or extensively studied. For example, if the 77% incidence of basophillic granular organisms in the gills of the Carson coho salmon does represent an infestation of microsporidian protozoan parasites, is the intensity of infestation severe enough to cause irrepairable damage that might af feet survival?

The results of the viral assays are questionable because the Rangen Laboratory is the only one that found evidence of viruses in these stocks (however, the veterinary pathologist did find evidence of a pox-type virus in one kidney from the Kooskia Hatchery spring chinook salmon). Secondly, even if the virus identification were substantiated, we cannot be sure of the significance of positive test results.

However , this variation is food for thought in how survival of individual stocks might be improved in the future through a closer examination of dietary and environmental requirements, and monitoring the incidence of sub-clinical diseases by random sub-sampling of populations.

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APPENDIX A

THE SURVEILLANCE OF VIRUS DISEASES IN SELECTED HATCHERY STOCKS OF SALMON AND STEELHEAD SMOLT IS THE COLUMBIA RIVER BASIN DURING 1978

CONTRACT No, 2-78
(EFFECTIVE FEBRUARY 1, 1978 TO SEPTEMBER 30, 1978)
USDC/NOAA PRUCHASE ORDER No, 02-78-M02-00189

THE SURVEI LLANCE OF VIRUS DISEASES IN SELECTED HATCHERY STOCKS OF SALMON AND STEELHEAD SMOLT IN THE COLUMBIA RIVER BASIN DURING 1978

FINAL REPORT PREPARED FEBRUARY 1, 1979

FOR

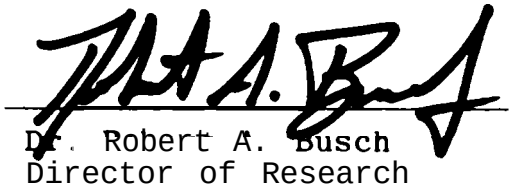
NATIONAL MARINE FISHERIES SERVICE
P.O. Box 38
MANCHESTER, WASHINGTON 98353

BY

RANGEN RESEARCH LABORATORY
ROUTE 1, Box 264
HAGERMAN, IDAHO 83332

RANGEN, INC. CONSIDERS THIS REPORT AS SATISFACTION IN FULL OF ALL OBLIGATIONS UNDER CONTRACT NO. 2-78. WE HAVE APPRECIATED THE OPPORTUNITY OF BEING OF SERVICE TO YOUR ORGANIZATION AND HOPE THAT WE MIGHT HAVE THE OPPORTUNITY OF DOING SO AGAIN IN THE FUTURE.

ANY QUESTIONS WITH REGARD TO THE CONTENTS OF THIS AND RELATED REPORTS MADE UNDER CONTRACT NO. 2-78 SHOULD BE DIRECTED TO:



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Director of Research
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Hagerman, Idaho 83332
(208) 837-6192

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I. INTRODUCTION

In the spring of 1978, the National Marine Fisheries Service (NMFS) Manchester, Washington laboratory began a study to evaluate various factors pertinent to the successful smoltification, ocean survival, and adult return of selected anadromous salmonid fish stocks of hatchery origin in the Columbia River basin. A portion of this study was devoted to ascertaining the general health profile of each stock at the time of smoltification and immediately prior to hatchery release andnatural Out-migration into saltwater. The purpose of this general health profile was to determine the occurrence and incidence of selected infectious diseases known to be potentially important to the growth and survival of salmonid fishes in general. The health profile data acquired can then be used in the evaluation and interpretation of other data obtained having to do with the relative success of saltwater adaptation, survival, and hatchery return potential.

Migrant hatchery fish stocks were examined for selected viral, bacterial, and parasitic disease agents using a wide variety of techniques as described in the parent NMFS project report. This report concerns itself only with the work contracted by NMFS to the Rangen Research Laboratory under Rangen Contract #2-78 (USDC/NOAA Purchase order So. 02-78-MOZ-00189) and titled THE SURVEILLANCE OF VIRUS DISEASES IN SELECTED HATCHERY STOCKS OF SALMON AND STEELHEAD SMOLT IN THE COLUMBIA RIVER BASIN DURING 1978. It pertains to that portion of the general health profile dealing with the screening of selected populations for the presence of important viral pathogens known to infect salmonid fish in North America. Included among these pathogens are infectious pancreatic necrosis (IPVN), infectious hematopoietic necrosis virus (IHVN), and Herpesvirus salmonis as well as any additional agents capable of inducing specific cytopathic effect (CPE) under the given conditions of surveillance.

The virus disease most commonly associated with Pacific salmon and steelhead trout stocks in the Pacific Northwest is infectious hematopoietic necrosis virus (IHVX). IRVN was first recognized in 1951 in sockeye salmon (Onchcrhynchus *nerka)* at Leavenworth Hatchery and kokanee salmon (landlocked sockeye salmon) at Winthrop Hatchery. The virus was isolated for the first time from sockeye salmon in 1958 (Wood, 1974). The disease is known to cause significant mortality in hatchery populations of Pacific salmon and steelhead trout as well as other salmonid species all along the Pacific coast of North America from California to Alaska and is considered endemic to many watersheds including the Columbia River system.

IHVN infection results in a peracute to acute course of infection resulting in high levels of mortality in chinook salmon (Onchorhynchus tshawytscha) and an acute

to subacute infection of sockeye salmon. The disease is characterized, as its name implies, by destruction of hematopoietic tissues resulting in an acute anemia, hemorrhage, and often severe mortality among fry and fingerling fish under hatchery conditions. Tnfcction seems to be primarily verticle in nature with the disease being transmitted vertically with the eggs and reproductive fluids of asymptomatically infected returning broodstock. The coho salmon (Onchorhynchus tshawytscha) appears to be more resistant to IHVN infection than the other species of Pacific salmon but can function as an asymptomatic carrier in the maintenance and dissemination of the disease (Wolf, 1972).

Survivors of an epizootic infection of IHNV are known to carry the virus in an eclipse phase typical of the rhabdovirus group of which it is a member. During this asymptomatic carrier state, the virus is not detectable by present methods of surveillance and is presumed to be non-infectious. This period of subclinical infection includes the time of physiological transition to saltwater known as smoltification. The latent effects of its presence upon smoltification, ocean survivial, and adult return are not known at this time. However, the detectable infectious virus has been shown to reappear immediately prior to spawning in infected populations of returning adults and is readily transmitted to the progeny at this time.

The early epizootiology of suspected viral diseases including IHNV in the Columbia River drainage is reviewed by Parisot et al. (1965). Several extensive surveys to determine the incidence and distribution of the virus have been conducted since that time in selected stocks of Columbia River trout and salmon. In 1972, Amend and Wood reported than no IHNV could be found in Columbia River stocks of Pacific salmon returning to 15 selected hatcheries in the State of Washington. These findings were based upon the extensive sampling of 130 to 150 ovarian fluid samples taken from each population at the time of spawning. Tebbit and McMichael (1973) found no evidence of IHNV in 10 Columbia River salmon stocks returning to hatcheries in the State of Oregon during 1971 and 1972. However, in 1973 they reported the confirmed isolation of IHNV from an adult spring chinook salmon stock returning to Oregon's Pelton Dam Holding Facility. Numerous additional studies have been undertaken by various state and federal agencies in subsequent years but have often failed to be comprehensive in design and execution and their results are often not readily available.

Even though IHNV has not been found to be a major problem for Columbia River stocks of chinook salmon, in general, it does continue to pose a threat to this species as well as sockeye salmon under hatchery condi-

tions. The real occurrence and incidence of IHNV is still not well defined due to problems in the detection of asymptomatic carrier states of infection during the eclipse stage. Knowledge is also lacking on the possible residual effects of the carrier state infection on successful smoltification, saltwater survival, and adult return. The disease remains endemic to the watershed and has been increasing in its known host range, geographical distribution and physical tolerances such as temperature.

Another salmonid virus, Infectious Pancreatic Necrosis Virus (IPNV), has not often been associated with or considered a problem in Pacific salmon stocks, IPNV is known to be endemic in the Columbia River drainage but is primarily recognized as a reovirus disease of trouts and chars. It is also known to be capable of infecting Atlantic, coho, and chinook salmon (Fish Health Section. 1974).

IPNV is considered to be the same disease originally described by M'gonigle (1941) in brook trout (salvelinus fontinalis) and Atlantic salmon (Salmo aslar) in hatcheries in the Canadian maritime provinces and later confirmed by MacKelvie and Artsob (1969). The disease was first isolated in the Western United States in 1963 by Parisot, et al.

IPNV disease is typically characterized by a peracute to acute course of infection and mortality of fry and fingerling fish or a subacute to chronic infection of larger fish up to and including yearling sizes. The infectious agent is readily transmitted horizontally through the water between fish by means of infected feces and urine from clinically diseased or asymptomatically infected carrier fish. It is also readily transmitted in a vertical manner from asymptomatically infected adult fish to their progeny via infected reproductive fluids and eggs. Survivors of an epizootic infection often remain asymptomatic carriers for life and continually shed the infectious virus into the water. The disease has been shown to be transmitted in the natural environment to susceptible stocks and maintained in wild and feral populations for extended periods of time with the incidence of infection gradually decreasing over time when no new introduction is made (Yamamoto, 1975). Sonstegard (1970) also showed long term survival of IPNV in the gastrointestinal tracts of selected non-salmonid fishes, picivorous birds, and aquatic invertebrates.

Until 1968, IPNV has not been known to occur in any of the Pacific salmon species. However, in July of that year, Wolf and Pettijohn (1970) isolated the virus from coho salmon fingerlings at Lamar National Fish Hatchery in Pennsylvania. The eggs of the infected stock had been taken from a spawning population of landlocked adult salmon in Lake Michigan. The fish had been checked for virus as

fry and were determined to be free of the infection. However, IPNV was enzootic among hatchery stocks of trout at the time and the virus was soon isolated from the fingerling salmon during an epizootic of furunculosis *(Aeromonas salmonicida).* No mortality could be attributed to the presence of the virus and evidence indicated that infection was due to horizontal rather than vertical transmission.

Amend and Wood (1972) surveyed 15 Columbia River stocks of Pacific salmon returning to hatcheries in the state of Washington in the fall of 1970, selecting only for IHNV on primary screen and confirmatory cultures. Consequently, no IPNV was reported. In 1973, Tebbit and McMichael reported on the surveillance of selected Columbia River stocks of Pacific salmon returning to selected hatcheries in Oregon. Their comprehensive design included the examination of visceral tissues and ovarian fluids from 60 adult females from each of four discrete populations. Adult fish and progeny fry were also bled and the sera examined for specific neutralizing antibodies against the common virus diseases. IPNV virus was isolated from two of twelve five-fish tissue pools from adult coho salmon returning to the Bonneville hatchery in 1971 and also from their progeny fry in thirty of thirty ten-fish pools indicating vertical transmission. It is interesting to note that McMichael (1974) was only able to isolate virus from the fry progeny at 30 and 60 days of age post hatch and that these same fish as fingerlings, when sampled at 90 and 210 days post hatch, no longer yielded detectable levels of virus but did demonstrate specific neutralizing antibodies against IPNV as titers in excess of 1:200 evidencing prior exposure. Attempts as horizontal transmission of the virus under laboratory conditions were unsuccessful at 15 C.

A report in the FAO Aquaculture Bulletin (1973) indicated that McMichael's coho isolate was unlike the ATCC UR-299 (American Type Culture Collection) IPNV trout isolate and more typical of French isolates of IPNV that loose 99% of their infectivity in a single freeze/thaw cycle.

When Tebbit and McMichael (1973) continued their sampling program, they found returning adult populations and their fry progeny at all of the selected hatcheries sampled were negative for IPNV in 1972 and 1973. However, they were able to demonstrate specific neutralizing antibodies in the sera of 270 adult fall chinook and coho salmon. The overall incidence was 74% in the returning coho and 92% in the returning fall chinook populations. In 1972, they were also able to isolate IPNV from adult coho salmon returning to Cascade Hatchery but the virus could not be demonstrated in the fry progeny nor in the adult returns the following year. Tebbit and McMichael (1973) also reported finding IPNV in adult chinook salmon returning to the Pelton Dam Holding Facility in 1973 after

the adult returns and progeny were found to be free of the virus in the 1971 and 1972 broodyears.

IPNV was isolated from steelhead trout being reared at Idaho Power Company's Niagria Springs Steelhead Hatchery in the Snake River canyon of Southern Idaho near Buhl in 1974. IPNV is known to be endemic to the commercial rainbow trout hatcheries in the local area and its appearance at the Niagria Springs station was not surprising. The virus has since reappeared at the station periodically and has been associated with significant mortality.

In 1974, Wood indicated that he had yet to come up with positive IPNV isolations from Columbia River stocks in Washington state. However, in 1975 Tebbit reviewed McMichael;s initial surveillance results and continued the program. Again looking at visceral tissue samples, ovarian fluids, and progeny fry, Tebbit was able to demonstrate IPNV in six of twelve five-fish tissue pools from adult coho salmon returning to the Cascade Hatchery during the 1972 broodyear, but he could not demonstrate verticle transmission to the progeny fry. That same year Tebbit also isolated IPNV from a population of spring chinook salmon fry at the Pelton Dam Holding Facility that were the progeny of an adult stock that had been diagnosed with a confirmed asymptomatic infection of IHNV (Tebbit, 1975). During the 1973 broodyear, Tebbit was able to detect IPNV specific neutralizing antibodies in the sera of adult coho salmon returning to the Sandy Hatchery and in the sera of adult coho salmon returning to the Bonneville Hatchery. None of these seropositive populations nor their fry progeny yielded a confirmed viral isolation. Tebbit's continued surveillance during the 1974 broodyear failed to yield virus from any of the adult returns sampled but IPNV specific neutralizing antibodies were found in a stock of landlocked spring chinook salmon in the Detroit River impoundment on the North Santiam River in Oregon. An endemic infection of IPNV had previously been demonstrated in wild cutthroat trout populations in these same waters.

Based upon the finding of IPNV and IHNV in stocks of anadromous salmonids in the Columbia River basin, the state of Oregon established a management policy prohibiting the transport of any Columbia River stocks of fish to a coastal river system for fear of disseminating an endemic viral disease problem.

In 1975, Mulcahy and Sanders reported isolating IPNV from spring chinook salmon at the Oregon Fish and Wildlife Commission's Corvallis Research Laboratory once again docementing the fact that IPNV can indeed infect Pacific salmon and steelhead trout. It is becoming recognized that IPNV may be all too common as an asymptomatic infection in a wide diversity of discrete spawning populations in the Columbia River basin and that it may not be easily recognized or clinically diagnosed in certain types of samples at certain stages in the life history of the Pacific salmon and

steelhead trout. It still remains to be shown whether or not the presence of an endemic infection of IPNV has a detrimental effect upon the population. Mortality directly attributed to the presence of the virus under hatchery conditions has yet to be demonstrated for Pacific salmon stocks but is know to occur with steelhead trout populations.

The last of three salmonid viruses to be found in the Columbia River basin is *Herprresvhus salmonis.* This virus or other closely related viruses are known to infect rainbow trout *(Salmo* gairdneri) and sockeye salmon at all stages in their life history and has been implicated in mortality of both fry and adult fish. The virus was first described in Japan as being endemic among certain stocks of sockeye salmon. It has only been diagnosed once in the United States as an asymptomatic infection of rainbow trout brood-stock at the Winthrop National Fish Hatchery. The virus still remains a fairly unknown entity with regard to the general health of Pacific salmon and steelhead trout stocks as its true host range, geographical distribution, and general ecology and epidemiology has yet to be determined (Wolf, et al., 1975).

II. MATERIALS AND METHODS

Procedures of viral surveillance were designed to fit within the overall field sampling program and budget of the parent study and give optimum sensitivity and accuracy with regard to detecting the presence of any of the three major salmonid viruses found within the Columbia River basin.

A total of 28 stocks of chinook salmon, coho salmon, and steelhead trout from 18 different Columbia River basin hatcheries (Figure 1 and Table 1) were examined for the presence of virus at the time of smoltification and hatch-ery release during 1978. Field sampling was conducted by NMFS personnel according to procedures established by and with field sampling kits provided by the Rangen Research Laboratory (Appendix A). Most of the hatchery stocks being sampled were first transported in a live haul container back to the NMFS Laboratory in Manchester, Washington. They were then maintained in freshwater flow-through systems (Beaver Creek water supply) for one to two days prior to sampling and saltwater introduction. A few stocks were sampled at the site of the production hatchery prior to transport back to the Manchester Laboratory. The field sampling diluent provided was formulated (see Appendix B) to provide optimum survival of any infectious virus under transport conditions while inhibiting the growth of any microbial contaminants. It was provided in sterile graduated polycarbonate screw-cap tissue culture grade centrifuge tubes (Corning #25310, Corning Glass Works, Corning, New York). The use of the graduated tube allowed

FIGURE 1. GEOGRAPHIC LOCATION OF SELECTED COLUMBIA RIVER DRAINAGE SALMON AND STEELHEAD HATCHERIES SAMPLED FOR VIRUS DURING 1978.

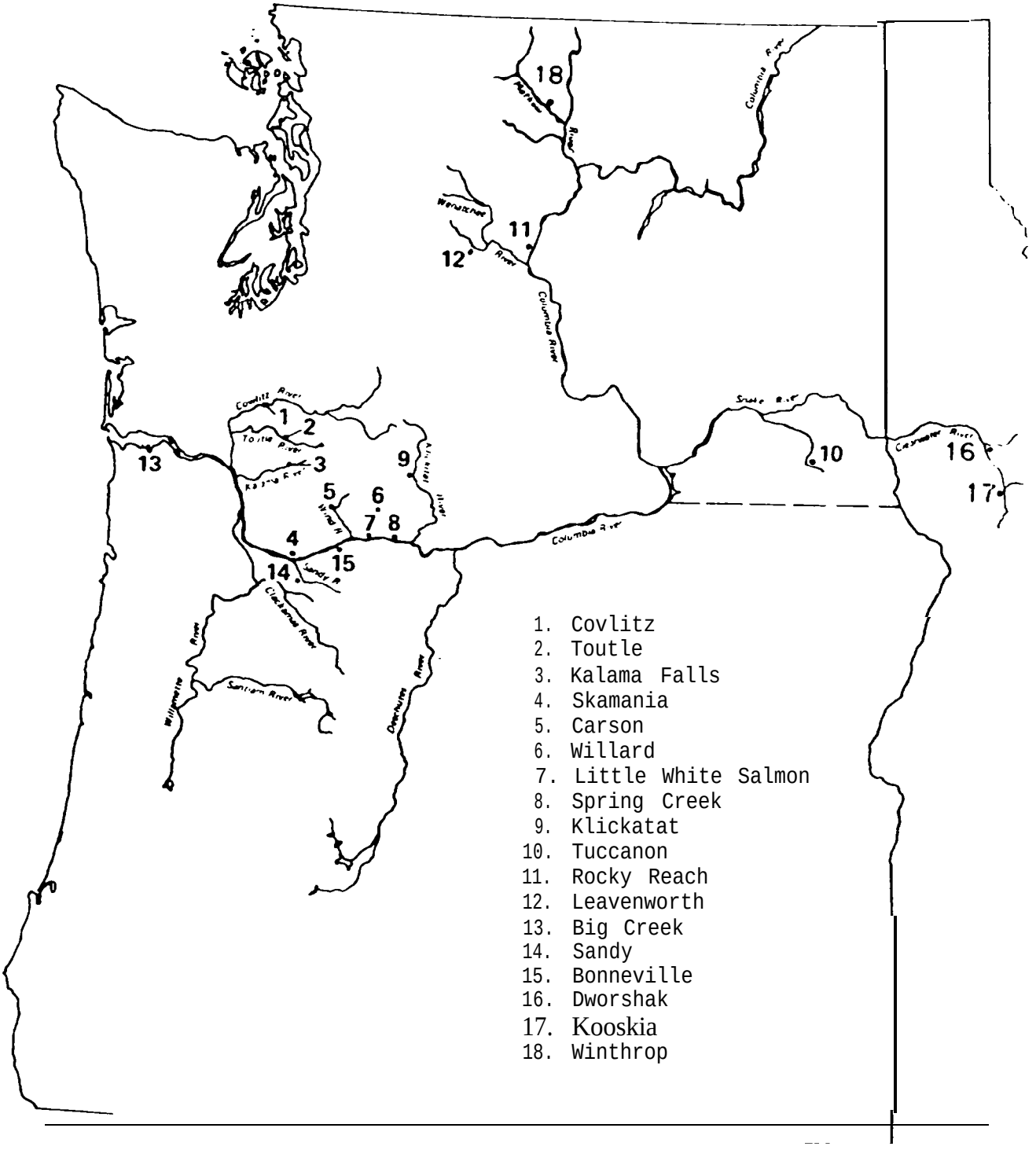

TABLE 1, STOCKS OF PACIFIC SALMON SMOLT EXAMINED FOR VIRUS AT THE TIME OF RELEASE FROM SELECTED COLUMBIA RIVER DRAINAGE HATCHERIES DURING 1978.

STATION	STOCK EXAMINED	DATE SAMPLED	RANGEN ACC. SO.
Big Creek	coho salmon	5/9/78	177-78
	coho salmon (Cowlitz stock)	5/9/78	**178-78**
Bonneville	fall chinook salmon (group I)	5/26/78	216-78
Carson	coho salmon (yearling)	4/26/78	146-78
	spring chinook salmon	5/2/78	158-78
Cowlitz Salmon	fall chinook salmon	**6/19/78**	**269-78**
Dworshak	steelhead trout	*4/26/78*	147-78
Kalama Falls	spring chinook salmon	*3/3/78*	**070-78**
	coho salmon	*5/6/78*	168-78
	fall chinook salmon	**7/11/78**	**197-78**
	fall chinook salmon	**9/15/78**	**356-78**
Klickatat	coho salmon	5/1/78	153-78
Kooskia	spring chinook salmon	**4/25/78**	148-78
Leavenworth	spring chinook salmon	**4/22/78**	138-78
	steelhead trout	**5/3/78**	**162-78**
Little White Salmon	fall chinook salmon	**5/25/78**	**115-78**
Rocky Reach	coho salmon	5/4/78	**161-78**
Sandy	coho salmon	*5/7/78*	165-78
Spring Creek	fall chinook salmon	**3/21/78**	099-**78**
Skamania	steelhead trout	*5/7/78*	**166-78**
Toutle	coho salmon	*5/3/78*	**159-78**
	coho salmon (Montlake stock)	**5/18/78**	**199-78**
	fall chinook salmon	**6/9/78**	*250-78*
Tuccanon	steelhead trout	*5/16/78*	189-78

TABLE 1. (CONTINUED)

Willard	coho salmon (group II)	5/25/78	214-78
	coho salmon (group III)	6/13/78	249-78
	fall chinook salmon	7/12/78	298-78
Winthrop	steelhead trout	5/5/78	167/78

for an accurate sample dilution factor calculation based upon the displacement method as 0.2 g of tissue was pooled from each of five fish into 9 ml of field sampling diluent and a total of twelve five-fish pools collected from the randomly selected 60 fish sample.

Following completion of all sampling procedures and the Field Sampling Data Sheet as outlined in Appendix A, the complete refrigerated field kits were returned to the Rangen Research Laboratory in Hagerman, Idaho via Grayhound NBO Package Express service. The average time in transit was 20 hours and the temperature of the samples upon receipt was consistantly between 6 C. and 8 C.

Upon receipt into the Rangen Research Laboratory, the receiving temperature of the samples was recorded. The twelve sample tubes were assigned a group accession number and each tube in the group given a serial numeric identifying code. The refrigerated tissue pools were homogenized in *situ* with a Polytron PUC-2-110 homogenizer fitted with a steam sterilizable stainless steel PT-10 generator (Brinkman Instruments, Westbury, New York) for 30 seconds. The homogenized tissue samples were then centrifuged at 2,000 X g for ten minutes at 4 C. in a Sorval RC-5 refrigerated centrifuge with an HS-4 rotor and appropriate tube adapters (Dupont/Sorval, Newtown, Conn.) to remove large cellular debris. One half milliter of the supernatant was pipetted off with a sterile disposable polyethylene tip and diluted in 2 ml of a disinfecting diluent (Appendix B) and incubated at 4 C. overnight for decontamination from fungal and bacterial agents. This procedure resulted in a final 1:50 working dilution of the original tissue sampled. All samples were maintained at 4 C. during all preparatory procedures.

Samples received into the laboratory were screened for the presence of virus within seven days of sampling. Viral screen tests were conducted with a microculture system using the Chinook Salmon Embryo (CHSE-214) cell line in passages 268 through 279 and a MEM-10-Tris-PSF media (Appendix B) at 12.5 C. incubation for 21 days. Four replicate wells of each of two dilutions were run for each of the pooled tissue samples. Microculture screen tests were prepared by dropping 0.05 ml of the decontaminated and diluted 1:50 tissue pool into each of the first four wells of an eight well series on a sterile Microtest II (Falcon #3040, Becton, Dickinson, and Co.) tissue culture plate and 0.025 ml of the same sample into the last four wells of the eight well series using an Oxford 8000H sampler and a sterile Oxford 810S tip. During preparation, the microculture plates were temporarily covered with a Falcon #3041 sterile lid andmaintained at 15 C. Appropriate IPNV and IHNV positive controls and negative media and cell controls were set up for each group tested. After the sample dilutions had been delivered to the microculture plate, a stock flask of CHSE-214 cells that had been grown to 95% confluence at 20 C. in MEM-10-Tris-PSF was

was examined under the inverted microscope for confluence and quality and dissociated in 4 ml of PAN/EDTA media (Appendix B). The PAIG/EDTA cell suspension was centrifuged at 1500 X g for 4 minutes. The supernatxnt was decanted and the pelleted cell mass resuspended in a small volume of MEM-10-Tris-PSF media. The resuspended cells were further diluted in MEM-10-Tris-PSF media to a final 1:4 working concentration based upon the surface area split ratio and placed in a sterile 100 ml covered flask containing a sterile magnetic stir bar. The diluted cell suspension was placed on a refrigerated magnetic stir plate with slow stirring to maintain the cells in suspension. A sterile Minipet repipetting syringe (Manostat #71-5000-010, hlanostat, New York, N.Y.) was fitted with a sterile disposable 18g X 1½" hypodermic needle and primed with the chilled CASE-214 cell suspension. Fifteen hundredths of a milliliter of the diluted CHSE-214 cell suspension was pipetted into each well on the inoculated microculture test plate. The plate was immediately sealed with Falcon #3044 pressure sensative film and incubated at 12.5 C. The suspended CHSE-214 cells were allowed to settle down through the sample material and attach to the bottom of the well.

All tests were incubated at 12.5 C. for 21 days. All wells of both test and control series were periodically read under an inverted microscope for evidence of specific cytopathic effect (CPE), cytotoxicity, and microbial contamination. The daily observations were recorded on the Virological Examination Report Sheet (Appendix C). If more than four wells in an eight well series were found to be cytotoxic, the original decontaminated sample was diluted 1:2 with disinfecting diluent to a final working dilution of 1:lOO and re-run on the microculture screen procedure as described above. If more than four wells in an eight well test series was found to be contaminated, the original sample was filter sterilized through a 0.45u membrane filter and re-run on the microculture screen procedure. If any of the wells in the control series demonstrated any unusual or inconsistat results at any time during the 21 day incubation period, the microculture screen procedure was repeated with the original samples.

At the end of the 21 day incubation period, the 96 wells on each plate (8 wells for each of 12 pooled tissue samples in the lot) were classified as being either positive (definite CPE), questionable (possible CPE or cytotoxicity), or negative (no evidence of CPE of cytotoxicity) and pooled into one of three tubes according to its classification. After the supernatant media had been pooled from the microculture screen plates for further testing, all wells of the plate were stained with a 1% alcoholic solution of crystal violet and dried as a permanent record of the screen results.

The presence or absence of virus in each of the three classified screen pools was confirmed and identified by means of a microculture serum neutralization procedure. One

quarter of a milliliter of sterile field sampling dilucnt was placed in each of the first three wells of an eight well series on a sterile microculture plate. Twenty five thousandths of a milliliter of a 1:lOO working dilution of EFDL =149 Polyvalent IPNV Antisera (Eastern Fish Disease Laboratory, Kearneysville, West Virginia) was pipetted into the nest two wells of each eight well series. Twenty five thousandths of a milliliter of a 1:lOO working dilution of EFDL =150 IHN Antisera was pipetted into the next two wells of' each eight well series and finally 0.025 ml of a 1:lOO working dilution of EFDL =100 normal rabbit sera was pipetted into the last well of each eight well series. Each of the three classified and pooled screenmaterialswere then diluted to 10^{3} by pipetting 0.025 ml of the material into the first well of an eight well series, mixing, and transfering 0.025 ml into the second well, mixing, and transferring 0.025 ml into the third well and mixing. Twenty five thousandths of a milliliter of the 10^{-3} dilution of the sample in the third well of each series was then transfered to each of the remaining five wells in the series. Appropriate positive IPNV and IHNV controls as well as negative media and cell controls were prepared in the same manner. The prepared microculture serum neutralization plates were then covered temporarily with a sterile lid and incubated at 15 C. for 60 minutes to affect appropriate neutralization of any virus present. CHSE-214 cells were then prepared to a 1:2 dilution based upon surface area as described above and 0.15 ml of the diluted cell suspension pipetted into the last five wells of each eight well test series on the plate. The plate was immediately sealed with film and incubated at 12.5 C. for five days.

The serum neutralization results were read under an inverted microscope at the end of the five day incubation period. The results were recorded on the Virological Examination Report Sheet (Appendix C). Destruction of the cell monolayer with characteristic CPE in well four, five, and eight of a test series indicated the confirmed presence of IHNV in that sample pool. Distruction of the cell monolayer with characteristic CPE in wells six, seven, and eight indicated the confirmed presence of IPNV in that sample pool. Distruction of the cell monolayer with characteristic CPE in wells four, five, six, seven, and eight could indicate the presence of Herpesvirus salmonis, a mixture of viral agents, or partial neutralization of a particular strain of a virus in which case additional procedures would have been applied to confirm identification. All wells demonstrating CPE in an eight well series on the serum neutralization plate were pooled and lyophilized in a stabolizer as a reference stock culture. All wells of the serum neutralization plate were then stained with a 1% alcoholic solution of crystal violet and dried as a permanent record of the results.

III. RESULTS

A total of 336 Pacific salmon and steelhead trout smolts representing 28 discrete anadromous stocks at 18 Columbia River basin hatcheries were tested for the presence of infectious viruses during 1978. The results of these tests are summarized in Table 2 for each of the hatchery stocks examined. Neither infectious hematopoietic necrosis virus (IHNV) nor Herpesvirus salmonis were identified during the course of the study.

Infectious pancreatic necrosis virus (IPNV) was confirmed at 12 of 18 hatcheries sampled and in 16 of 28 stocks of smolted fish examined as summarized in Table 3. IPNV was most commonly found in populations of coho salmon (82% incidence among populations sampled) and least likely to occur in populations of fall chinook salmon smolt (25% of the populations examined). The incidence of infection among five-fish sample pools within each 60 fish lot was the lowest for fall chinook salmon smolt with an average of 12.5% in the two infected populations examined. The same measure of carrier incidence for infected spring chinook salmon smolt populations was 75% 64% for infected coho salmon smolt populations and 61% for infected steelhead trout populations. None of the confirmed IPNV infections could be associated with any significant mortality or loss in any of the populations.

IV. DISCUSSION

The failure to isolate either Herpesvirus *salmonis* or infectious hematopoietic necrosis virus (IHNV) during the course of this study is not unusual when considered within the given limitations of the experimental design applied. *Herpesvirus salmonis* or other closely related salmonid viruses have yet to be isolated from coho salmon, chinook salmon, or steelhead trout In the United States. In fact, the virus has only been isolated once from a Columbia River basin salmonid stock and has not been seen since. Due to the fact that our knowledge of the disease is still limited in terms of its occurrence, incidence, and pathogenesis, it can only be said that the virus was not detected within the limits of the experimental design. Possibly by broadening the scope of the study to include conditions known to be optimum for the recovery of the virus at all stages in the life history of Pacific salmon and steelhead and the application of more sensative techniques of serological surveillance such as detection of specific neutralizing factors in the sera or other body fluids or the detection of specific antigens or antibodies in the various body tissues and fluids by means of enzyme linked immunoadsorbent assay (ELISA) or counterimmunoelectrophoresis (CIE) procedures could also

TABLE 2, SUMMARY OF PRELIMINARY SCREEN RESULTS AND CONFIRMED IDENTIFICATION OF VIRUS ISOLATED FROM SELECTED COLUMBIA RIVER DRAINAGE HATCHERY STOCKS OF ANADROMOUS SALMONID SMOLTS DURING 1978,

STATION	STOCK	NUMBER OF SCREEN POOLS			CONFIRMED RESULT
			SUSPECT	NEGATTVE	
Rig Creek	coho salmon	6	0	6	IPNV
	coho salmon (Cowlitz stock)	4	5	3	IPNV
Bonnevl lie	fall chinook salmon (group T)	12	0	0	negative
Carson	coho salmon (yearling)	3	3	6	IPNV
	spring chinook salmon	0	3	9	negative
Cowlitz Salmon	fall chinook salmon	1	2	9	negative
Dwarshnk	steelhead trout	0	a	4	TPNV
Kalama Falls	spring chinook salmon	0	0	12	negative
	coho salmon	12	0	0	IPNV
	fall chinook salmon	12	0	0	negative
	fall chinook salmon	3	3	6	TPNV
Kl ickatat	coho salmon	4		4	IPNV
Kooskia	spring chinook salmon	6	1	5	TPNV
leavenwor th	spring chinook salmon	12	0	0	OPNV
	steelhead trout	9	1	2	ncgntive
li ttle White Salmon	fall chinook salmon	12	0	0	negative
Rocky Reach	coh salmon	0	0	12	negative

TABLE 2. (CONTINUED)

Sandy	coho salmon	12	0	0	IPNV
Spring Creek	fall chinook salmon	4	5	3	negative
Skamania	steelhead trout	12	0	0	IPNV
Toutle	coho salmon	9	1	2	IPNV
	coho salmon (Montlake stock)	12	0	0	IPNV
	fall chinook salmon	0	1	11	TPNV
Tuccanon	steelhead trout	0	10	2	negative
Willard	coho salmon (group II)	12	0	0	negative
	coho salmon (group III)	7	0	5	IPNV
	fall chinook salmon	12	0	0	negative
Winthrop	steelhead trout	10	1	1	IPNV

TABLE 3, INCIDENCE OF INFECTIOUS PANCREATIC NECROSIS VIRUS IN SELECTED STOCKS OF ANADROMOUS SALMONID SMOLTS AT COLUMBIA RIVER DRAINAGE HATCHERIES DURING 1978,

	TOTAL STOCKS EXAMINED	STOCKS WITH CONFIRMED IPN	ISCIDENCE OF INFECTION
A11 Stations Sampled	18	12	67%
All Stocks Combined	28	16	57%
Coho Salmon	11	9	82%
Fall Chinook Salmon	8	2	2 5%
Spring Chinook Salmon	4	2	50%
Steelhead Trout	5	3	60%

give a better understanding of the ecology of the disease and its impact on anadromous stocks of salmonids.

Infectious hematopoietic necrosis virus (IHNV) is known to be endemic to the Columbia River basin but is primarily considered a disease of fry and fingerling chinook salmon and steelhead trout. Coho salmon appear resistant but can function as asymptomatic carriers. IHNV is known to enter into a non-infectious eclipse phase of infection in post-epizootic or convalescent populations and, as such, cannot be isolated and identified by routine methods of culture as applied in this study. In order to get a better understanding of the ecology and overall impact of IHNV on the anadromous salmonid stocks of the Columbia River basin, surveillance programs should include testing of ovarian fluids from adult spawning populations as well as progeny fry at the swim-up stage as these are the two stages in the life history of the fish when the virus is known to exist in the infectious form. Techniques of serological surveillance using serum neutralization, ELISA, and CIE procedures should be effective in detecting the virus at these and other stages when the virus may be present in the non-infectious eclipse form.

The isolation of infectious pancreatic necrosis virus (IPNV) from the stocks examined and, in particular, the finding of a relatively high incidence of the virus among the populations in comparison to previous surveillance studies seems unusual on initial examination. However, when one considers that IPNV is; 1)known to be endemic to large areas of the Columbia River basin; 2) known to infect both Pacific salmon and steelhead trout; and 3) has been previously detected in at least ten discrete populations of Columbia River salmon and steelhead at seven different stations since 1971, the results take on a more consistant image. It is also noted in this study and in several previous surveillance studies that IPNV has been isolated only from visceral tissues of infected returning adult stocks and their 30 to 60 day old progeny fry and not from reproductive fluid samples. However, our understanding of the epidemiology of IPNV in anadromous stocks of Columbia River salmon and steelhead trout seems to be primarily based upon studies which utilized ovarian reproductive fluid samples exclusively and were specifically designed to monitor for IHNV. When McMichael (1974) and Tebbit (1975) sampled both reproductive fluids and pooled visceral tissues from returning adult salmon populations,they were able to isolate IPNV from four discrete populations but all four IPNV isolates obtained came exclusively from pooled visceral tissue samples while all of the paired reproductive fluid samples remained negative for the virus (Tebbit, 1979, personal communication). This observation would indicate that surveillance studies based solely upon reproductive fluid samples, while being well suited to the detection of IHNV, may not accurately

reflect the true incidence of IPNV in a population of asympto matic adult carriers.

It should also be noted that McMicheal (1974) consistantly failed to isolate IPNV from known infected fry populations of Pacific salmon after 60 days of age post feeding. Consequently, surveillance programs based upon the sampling of fry and fingerling sized fish between 60 days of age and prior to smoltification may easily fail to detect the presence of IPNV.

IPNV as well as many other diseases of viral etiology are known to be stress mediated as well as species and age specific. The period of smoltification in the life history of an anadromous salmonid fish is recognized as a major time of physiological change and stress to the animal, particularly under hatchery conditions of intensive culture and nutrition. It is therefore reasonable to assume that this period of snoltification and physiological stress, particularly when coupled with major changes in hormonal balances and behavior, could well exacerbate a preexisting subclinical infection of IPNV or even increase susceptibility of an uninfected populat-ion to infection from an endemic source. To the best of our knowledge, this is the first surveillance study to report on results obtained specifically from populations undergoing smoltification.

Another consideration for discussion is the demonstrated low incidence of IPNV to be found among the asymptomatically infected populations. Tebbit and McMicheal (1974) reported only two of twelve five-fish tissue pools taken from adult coho salmon returning to Bonneville Hatchery in 1971 were found to be positive for IPNV. Only six of twelve pools of tissue from adult coho salmon returning to Cascade Hatchery in 1972 were positive for IPNV (Tebbit, 1975). In the present study, seven of the sixteen populations found to be infected with IPNV demonstrated fewer than half of the tissue pools to be positive for virus on initial screening. This finding would indicate that a full 60 fish sample, based upon hypergeometric sampling statistics, may be necessary in order to consistantly detect the virus. Spot checks of fewer than 60 fish are apt to miss an infection of low incidence.

A final consideration in the surveillance of IPNV in the Pacific salmon and steelhead trout stocks of the Columbia River basin is the demonstrated variability in the occurrence of the virus between different year classes of a particular stock. In extensive sampling between 1971 and 1974, McMichael (1974) and Tebbit (1975) consistantly failed to isolate IPNV from the same hatchery stocks during subsequent years even though neutralizing antibodies indicating prior exposure were at times found to be present. The presence or absence of IPNV in a particular year class of a given stock of fish may not necessarily mean that all other year classes of that same stock will be similiarly infected or free of the disease.

Based upon these observations including the relatively low

incidence of IPNV both among and within populations of anadromous Pacific salmon and steelhead trout in the Columbia River basin, the serological findings of McMichael (1974) and Tebbit (1975) utilizing neutralizing antibody techniques, and the variability of clinical infection between the different life history stages and year classes of a particular stock, it is suggested that future attempts at viral surveillance include sampling of both visceral tissues and reproductive fluids from spawning populations. Progeny fry should be examined at 30 days of age post feeding and fingerling fish at a time just prior to hatchery release during smoltification. Attempts at isolation from the various stages in the life history should be coupled with techniques of serological surveillance based upon serum neutralization, ELISA, and CIE procedures. All year classes of a particular stock should be examined over the course of the study.

The demonstrated presence of IPNV in smolted stocks of Pacific salmon and steelhead trout in the Columbia River basin should be of concern but not alarm to resource management agencies. IPNV has yet to be associated with any significant mortality in stocks of Pacific salmon under hatchery conditions and is not an altogether common cause of mortality in stocks of steelhead trout with the possible exception of one station in Southern Idaho. The disease does, however, hold the potential for significant losses of steelhead trout under hatchery conditions and significant harm to this species. The sublethal impact of the virus on Pacific salmon throughout their life history, particularly with regard to ocean survival and adult return, has yet to be determined.

V. CONCLUSIONS

1. Infectious pancreatic necrosis virus (IPNV was isolated from 16 of 28 stocks of Pacific salmon and steelhead troutsmoltsat 12 of 18 Columbia River hatcheries in 1978.

2. The presence of infectious pancreatic necrosis virus (IPNV) in stocks of Pacific salmon and steelhead trout smolts at Columbia River basin hatcheries in 1978 was not associated with any significant mortality.

3. Infectious hematopoietic necrosis virus (IRNV) was not isolated from any of 28 stocks of Pacific salmon and steelhead trout smolts at 18 Columbia River basin hatcheries in 1978.

4. Herpesvirus salmonis was not isolated from any of 28 stocks of Pacific salmon and steelhead trout smolts at 18

Columbia River basin hatcheries in 1978.

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RANGEN RESEARCH HATCHERY

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HAGERMAN, IDAHO 83332

SHERIES DIVISION

RANGEN

NMFS - MIGRANT SMOLT HEALTH INDEX STUDY

-Field Sampling Procedures-

A. Field Sampling Kit - each field sampling kit consists of 13 graduated placstic screw-cap tubes of sterile viral sampling and transport diluent (12 sample tubes and 1 replacement tube), 1 sample tube shipping rack, 1 preaddressed shipping label for return of field sampling kit and samples, 1 instruction and field sampling data sheet, 1 insulated shipping container. This kit is sufficient for sampling a single lot of 60 fish for virus screening. Field kits should be stored at 4 C. prior to use and at no time should they be frozen or held above 15 C. Gel packs of refrigerant should be held in the freezer prior tc packing for return shipment.

B. Sampling Procedure - 60 fish should be randomly selected from a defined lot and divided into 12 5-fish pools. The fish should be sacrificed by a blow to the head and aseptically opened to expose the kidney and viscera with sterile dissection instruments. Care must be taken during dissection not to cut into the gastro-intestinal tract or otherwise contaminate the internal tissues. Tissues for viral assay should be quantitatively sampled with the modified Russion Tissue Forceps provided. The forceps should be dipped in 70% isopropyl alcohol and wiped clean to disinfect between 5-fish pools. Tissues from each fish are sampled by taking one (1) full forcep of material and depositing it in the sterile viral sampling diluent. This tissue volume is critical and the tissue should only fill the cups of the forceps, no more, no less. Tissues to be sampled from each of the 5 fish in the pool in order of sampling are the liver (being careful to avoid the gall bladder and the introduction of bile into the sample), the spleen (being careful1 to sample as little as possible of associated adipose or fat tissue), and the kidney. After all three tissues from each of the five fish in the pool are sampled and placed in the tube of sampling diluent, the tube is capped tightly and placed securely in the tube rack. Samples and diluent should be kept cool and out of the sun during the'entire sampling procedure.

C. Shipping - When all of the samples are taken and the field sampling data sheet has been completed, all materials are placed back into the insulated shipping container together with sufficient frozen gel pack refrigerant to last for 48 hours. The preaddressed shipping label is placed on the outside of the container

and the container sent as soon as possible after sampling the single lot. Shipment should be by either Grayhound Package Espress or United Parcel Service. Avoid shipping over weekends or holidays. If necessary, hold samples at 4 C. in a refrigerator and ship on the following Sunday or Monday.

D. If there are any questions or problems. contact:

Dr. Robert A. Busch
Rangen Research Station
Route 1, Box 264
Hagerman, Idaho 83332

Office: (208) 837-6192
Home: (208) 837-6370

VII. APPENDIX A

NMFS - MIGRANT SMOLT HEALTH INDEX STUDY

-Field Sampling Data Sheet-

NMFS Sample Code: _____ Date: ____/____/

Sampling Location: _____ Time: _____ hours

_____ Technician: _____

Species Sampled: _____

Original Source and Identification _____

_____

_____

Sampling Notations, Observations, Gross Lesions, etc.:

_____

_____

_____

_____

_____

_____

_____

Virus Disease History: Yes or No

1) Has a virus disease ever been diangosed in these fish stocks sampled? _____

2) Has a virus disease ever been diagnosed at the station of origin of these fish? _____

If the answer is "yes" to either or both of the above questions, please indicate which virus disease was diagnosed, on what date, and by whom:

_____

_____

_____

_____

_____

Sample Shipment Information:

Via: _____ Date: ____/____/

Point of Origin: _____ Time: _____ hours

_____

Sample Receipt Information:

Received Date: /____/____ Time: _____ hours

Condition: _____

Formulation of Medias and Reagents

A. Field Sampling Diluent

Dulbecco's PBS (10X stock)	
Gibco #408	100 ml
T/C Grade Water	800 ml
Gentamicin (50 mg/ml stock)	
Shering Corp.	4 ml
Amphotericin B (250 ug/ml stock)	
Gibco #529L	4 ml
Phenol Red (0.5% stock)	
Gibco #510	**4 ml**

Adjust pH to 7.2 with sterile 1N NaOH

Adjust final volume to 1 liter with T/C grade water

B. Decontamination Diluent

Dulbecco's PBS (10X stock)	
Gibco #408	100 **ml**
T/C Grade Water	**800 ml**
Gentamicin (50 mg/nl stock)	
Schering Corp.	**20 ml**
Amphotericin B (250 ug/ml stock)	
Gibco #529L	**2 ml**
Phenol Red (0.5% stock)	
Gibco #510	**4** ml

Adjust pH to 7.2 with sterile 1N NaOH

Adjust final volume to 1 liter with T/C grade water

C. MEM-10Tris-PSF Tissue Culture Media

Eagle/Earle MEM (Auto-Pow)	
Gibco #410-1700	4.701 g
T/C Grade Water	421.3 ml

Autoclave at 121 C. for 15 minutes

Cool and aspetically add:

Fetal Calf Serum (mycoplasma and virus free)	
Gibco #614	**50 ml**
1-Glutamine (200 mh!)	
Gibco f503	**5** ml
Sodium Bicarbonate Solution (7.5% stock)	
Gibco #508	**5** ml
Tricine Buffer (1M Tris)	
Gibco #573	**5** ml
Antibiotic-Antimycotic (100X stock)	
Penicillin 10,000 U/ml	

Streptomycin 10,000 ug/ml
Amphotericin B 250 ug/ml
Gibco #600-5240 **5** ml
Calcium Chloride (10% stock) 1 ml

Adjust pH to 7.2 with sterile 1N NAOH

Adjust final volume to 500 ml with T/C grade water

D. PAN/EDTA Dissociation Media

Versene (1:5000 solution)
Gibco #670-5040 100 ml
Pancreatin (4X N.F. reconstituted to 2.5% in 20 ml sterile T/C water)
Gibco #R13-5720-L **4 ml**
Phenol Red (0.5% stock)
Gibco #510 **0.4** ml

( ) Diagnosis
( ) Certification
( ) Surveillance

VIROLOGICAL EXAMINATION
FISH PATHOLOGY LABORATORY
RANGEN RESEARCH HATCHERY

File No.:____________

Acc. No.:__________

mpling Date:___/___/___ Time:__________hours Technician:________________

Receiving Condition: ( ) live ( ) fresh
( ) frozen ( ) refrigerated
Holding condition: ______hours _____ °C
Comment:

No. Sampled:

Lot #:				
Morb.:				
Mort.:				
Asyp.:				

Tissue Sampled: ( ) whole fish ( ) mixed viscera ( ) liver ____%
( ) spleen ____% ( ) pyloric caeca ____% ( ) kidney ____%
( ) ovarian fluids ( ) seminal fluids ( ) other:

Pooling:

Lot #	Fish/Pool	No. Pools	Lot #	Fish/Pool	No. Pools

Inoculant Preparation:

Pool #	Tis. wt. g.	Buffer ml	Sup.vol. ml	pen/strep ml stock	mycostat ml stock	gentamicin ml prep	filter 0.45 u	fina[illegible] fa[illegible]

Prep. Incubation: ____°C _____hours Inoculation Date:___/___/___ Time:____

thnician:________________ Inoc. Vol.: 0.025 0.05 0.1 0.2 ml ( ) wet ( ) [illegible]

( ) single ( ) duplicate ( ) microtiter ( ) multiwell ( ) 60 mm disl

Absorbtion Time: _____min. _____°C.

Cell Line	Passage	Seed Date	Vessel	Growth Temp.	Conf.	Quality	Inc. T[illegible]

Media:______________________ Control Virus:______________________

Lot #:______________________ ______________________

RESULTS:

Date	Day																																				

VIROLOGICAL EXAMINATION
(continued)

Serum Neutralization (microculture):

Set-up Date: / / Time: _____ hours Technician: _____

Sample Handling: ( ) raw ( ) filtered ( ) fresh ( ) stored 4 C. _____

Neutralizing Antisera: _____ Working Dil.: _____

_____ Working Dil.: _____

Control Sera: _____ Working Dil.: _____

Cell Line Passage Seed Date Vessel Growth Temp. Conf. Quality In

_____

Neutralization: Viral Sup. Working Dilution: _____ Volume: _____ml

Antiserum Volume: _____ml Ratio: _____

Incubation Time: _____min. Temp.: _____C.

Inoculation: Inoc. Vol.: _____ml ( ) wet ( ) dry Absorb. Time: _____mn

Media: _____ Lot #: _____

Control Virus: _____

Plate Identification: _____

ROW	Sample No.	WELL TEST	A	B	C	D	E	F	G	H	RESULTS
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											

Remarks:

Rangen Inc.
P.O. BOX 706 • 115 13 AVE. SO. • BUHL, IDAHO 83316 • (208) 543-6421

RANGEN RESEARCH STATION
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TELEPHONE (208) 837-6 192 LABORATORY
(208) 837-6191 HATCHERY

February 13, 1979

Mr. Tony Novotny
National Marine Fisheries Service
P.O. Box 38
Manchester, Washington 98353

Dear Tony;

Enclosed are two (2) copies of the final report on our contract #2-78 titled: The Surveillance of Viral Diseases in Selected Hatchery Stocks of Salmon and Steelhead Trout Smolt in the Columbia River Basin During 1978. I hope that it meets with your approval and is of pertinence to the parent study with regard to interpretation of the rest of the data. If you have any questions with regard to the study in general or this report in particular, please feel free to give me a call.

With regrad to the possibility of a major proposal to undertake a more complete study on the ecology and epidemiology of viral diseases, particularly IPNV, among Columbia River basin stocks of Pacific salmon and steelhead trout, I have mentioned basic considerations for its design in the discussion portion of this report and would be most happy to prepare a detailed proposal should a source of funding be available.

We have appreciated the opportunity of being of service to the National Marine Fisheries Service and your Laboratory during the course of this project. I hope that we have the opportunity of working together again in the future.

Sincerely,

Bob

Robert A. Busch, Ph.D.
Director of Research

APPENDIX B

HISTOPATHOLOGY REPORT

BIOMED RESEARCH LABORATORIES

BIOLOGICAL Testing - *Research & Development*

1115 E. Pike Street Seattle, Washington 98122 (206) 324-0380

October 24, 1978

National Marine Fisheries Service
Manchester Marine Research Station
Anthony Novotny
Fisheries Research Biologist
P.O. Box 38
Manchester, Washington 98355

SUBJECT: Six groups of salmon with 60 fish in each group formalin fixed.

METHODS AND MATERIALS:

Six groups of salmon with 60 fish in each group were received in formalin fixative following a 24 hour fixation in Bouin's fixative. Three blocks of tissues were parafin-embeded,sectioned and stained by standard methods.Block I1 contained the eye; block 12 the gill (generally the second gill arch) and block 13 the kidney and liver.Sections from each block were stained with hematoxylin and eosin, and a gram stain was made on the sections of liver and kidney. All blocks of tissue were prepared identically except for those of the eye.Initially,it was thought that only lesions within the eye itself would be found,and each eye was removed from the fish head before processing. It became apparent that there were important lesions adjacent to the eye,and the sections made late in the study also contained a full cross-section of the head to better study these lesions.

After scanning several groups of fish, a table of the lesions being found was established, and the lesions categorized by the severity. A minus (-) indicated no lesion, a plus (+) indicated the mildest recognizable lesion, two pluses (++ indicated intermediate severity and three pluses (+++) indicated the most severe lesion found. Gram stains were either positive or negative.

RESULTS: The lesions found frequently enough to warrent tabulation are identified on the table for each group of fish as follows: (A detailed description for each lesion is included later.)

1) Eye:

a. Myo - degenerative and regenerative lesions in the skeletal muscle adjacent to the eye.

b. Fat - fat necrosis and inflammatory inflitrates in the retrobulbar fat.

c. Misc- miscellaneous sporadic lesions.

2) Gill:

a. Lymph - increased numbers of lymphocytes.

b. Epith - Epithelial cell proliferation.

c. Misc - miscellaneous sporadic lesions.

3) Liver:

a. Fat - increased amount of fat in the liver

parenchyma,

b. KD - definite kidney disease w/gram plus bacteria.

c. Gran - granulomatous lesions w/out gram plus bacteria.

d. Misc- miscellaneous sporadic lesions.

4) Kidney :

a. KD - as in 3b.

b. Gran - as in 3c.

c. Ca - nephrocalcinosis.

5) Gram:

a.L - gram positive bacteria in vthe liver.

b. K -gram positive bacteria in the kidney.

A two page table for each 60 fish group identifies each fish and the severity of any lesions noted. At the bottom of each table is a total of the lesions found by severity.

A summary table presents the incidence of lesions within each group.It is expressed as a percent of the total fish in that group,and again broken down by severity of the lesions.

In general,it should be noted that these fish were well-fixed and provided excellent material for histological

study except when cross-sections of heads were made late in the study where intracranial hemorrhage was noted, perhaps as an artifact secondary to being “thumped” on the head during sampling.

Lesions from Block #1: The lesions in the skeletal muscle adjacent to the eye consisted primarily of irregular loss of myofibrills within muscle fibers. In cross-section, the sarcoplasm of affected fibers would be either granular or lost completely. In longitudinal sections of muscle, the muscle fibers would vary greatly in thickness with sudden abrupt loss of well-defined myofibrils. In one case (#4543 h’inthrop Steelhead) marked basophillia and increased numbers of muscle cell nuclei indicated an attempted regeneration. The amount of muscle available for evaluation varied from slide to slide; therefore, the estimation of severity is subjective, but the typical (+) lesion is a very mild one.

The lesions of the retrobulbar fat can be broken down into fat necrosis and actual inflammation (panniculitis). In fat necrosis, variable numbers of the fat cells would contain faintly eosinophillic, often granular deposits interpreted as the formation of soaps as the result of fat breakdown. The panniculitis consisted of cellular inflammatory infiltrates between fat cells. Monocytes and lymphocytes were the most typical inflammatory cells noted. These lesions were mild and over-lapping, and it did not appear important to tabulate them separately.

Miscellaneous lesions found in block #l from small numbers of fish included several inflammatory lesions of the eye itself. Chronic inflammation with mononuclear cell infiltrates were seen in the corneoscleral junction region of several fish,and a more widespread chronic inflammatory infiltrate was seen in the iris and urea1 tract (iridocyclitis) of one fish.Another fish had more widespread chronic ophthalmitis involving much of the globe. The optic nerves of several fish contained large numbers of mononuclear cells,another fish had focal mononuclear cell infiltrates in the choroid gland, and another fish had a chronic inflammatory infiltrate within the wall of a retrobulbar blood vessel.Intracranial hemorrhage or meningeal hemorrhage was noted in several fish.

Lesions from Block #2:In many section of gills, increased numbers of lymphocytes were noted. Small numbers of lymphocytes can be found beneath the gill epithelium of almost any salmon,typically in the areas where the gill filament joins the gill arch. In mild (+) cases, this lymphoid infiltrate increases to several cells in thickness, and in more severe (++ 4 +++) cases, the mass of lymphocytes may obliterate a portion of the space at the base of the gill filament and form recognizable collections of lymphocytes higher on the gill filament.

The gill epithelial proliferative lesion tended to parallel the lymphoid infiltrative lesions of the gill in severity.

The epithelial proliferation tended to develope on the tips of the gill filaments rather than adjacent to the gill arch, It also tended to develope on the surface of the filament between the secondary lamellae, In no case did this lesion progress to the point of causing fusion between adjacent gill filaments. Miotic figures could be found in the proliferating epithelium and often the areas of lymphoid infiltration and epithelial cell proliferation overlapped.

The most common sporadic lesion of the gills was found only in coho salmon. This lesion is identified as BGO in the tables because it presents as a basophillic granular mass of organisms up to 40 u in diameter located intracellularly or subepithelially on the gill filament between the bases of secondary lamellae without any obvious cellular reaction. This structure is gram negative, and may represent a microsporidian protozoan parasite.

In the spring chinook and Dvorshak steelhead, a large ciliated protozoan parasite was noted. This parasite varied in size up to approximately 150 microns, was oval to round in shape and was covered with cilia. It was found both free in the interfilamentous space and also embedded beneath a layer of proliferating epithelium. The Dvorshak and Winthrop steelhead also had microsporidian protozoan parasites, differing from the BGO in that they appeared to be surrounded by a more definite cyst wall, and the spores within the cyst

measured two to three microns in diameter. The numbers of these structures found was soo small to allow further study. Fish parasitologists are being consulted to help identify these parasites.

Miscellaneous non-protozoan gill lesions included occasional fish in which one to several secondary lamellae would have greatly dialated secondary lamellar capillaries. One fish had generalized capillary congestion of the gills. Another had sludging of granulocytic leukocytes in the secondary lamellar capillaries.

Lesions of the Liver: Fat deposition in the livers of these fish was uncommon, but when present was identified either as individual fat droplets in random hepatocytes, or as a descrete compact group of hepatocytes filled with fat vacuoles.

Grossly or histologically apparent lesions of corynebacterial kidney disease were uncommon in the liver, but when recognized consisted of areas of necrosis containing large numbers of small gram positive bacteria. Also, noted were several granulomatous lesions consisting of compact aggregates of macrophages organized into a granuloma, but without any detectable bacteria present. Much more common than either of the preceeding lesions was a focal accumulation of mononuclear cells (FM) in sinusoids or around portal tracts.

Lesions of the Kidney: The lesions of corynebacterial kidney disease and granulomas in the kidney were essentially as described for the liver. The abundant hematopoietic tissue made the granulomas more difficult to recognize. Also, occasionally areas of disrupted architecture and hemorrhage were seen. These lesions appeared to be secondary to the sampling of the kidney for the fluorescent antibody test for kidney disease bacteria and were not tabulated.

In one fish (#4025, spring chinook), the kidney contained a focal area of spindle-cell proliferation. These spindle cells appeared to be fibroblasts, and occasional cells within this area contained oval eosinophillic intracytoplasmic inclusion bodies approximately 50 microns in diameter.

Gram Stains: Interpretation of the gram-stained sections of kidney and liver is exceedingly difficult. Where the typical KD lesion is seen in the H & E section, gram positive bacteria are abundant and readily apparent. In sections without these lesions few definite bacteria could be recognized. Nuclear debri, cellular debri within kidney tubular lumens, and other small fragments of material tended to stain gram positive. The melamin granules of the macrophages also caused some difficulty in differentiating bacteria from other material.

In the discussion,suggestions for improving the accuracy of this proceedure will be discussed, Without this additional work,the percent of livers and kidneys harboring individual bacteria cannot be determined. An attempt was made to do so on the first three groups (Carson coho, spring chinook and

Dvorshak) but this data should not be entered as it is not significant (see discussion).

DISCUSSION:

A detailed statistical analysis was not attempted, being better left to the computerized data analysis. It should be noted that while the lesions were grouped into mild to severe categories, the (+++) category implies the most severe lesion found, but not necessarily that a severe lesion was found. In general, even the most severe lesions seen could not be interpreted as severe or life endangering. The significance of the data presented here will come when it is compared with data from other studies. Therefore, this discussion will deal primarily in generalities.

In the sections from block #I, the skeletal muscle lesions of the eye are interesting. The mildest (+) lesions possibly could be artifact of inadequate fixation. However, the more severe lesions do not appear to be artifact, and one case demonstrated regeneration, irrefutable evidence of muscle injury. Also, a more severe muscle lesion with frank necrosis and mineralization were recently found in salmon which had been in sea water approximately two months. This lesion was a more severe form of the same disease process seen in the fish of this study, again supporting the opinion that it is a genuine lesion. This lesion in a mammal would be strongly suggestive of a vitamin E/

selenium deficiency state, and has been reported to occur in fish although the known reports are not strongly documented. With regards to further studies, some consideration should be given to evaluating the vitamin E/selenium status of these fish. With respect to the differences between the groups, the steelhead fish have a higher incidence of these myodegenerative lesions.

The lesions in the fat also could have a pathogenesis involving vitamin E/selenium, similar to the steatitis or "yelllow-fat" disease of mammals. For adequate study of both the skeletal muscle and fat lesions in the future, cross-sections of the head will be made to include the eyes, brain and structures adjacent to the eyes. No parasitic lesions were found in the eyes, and only sporadic mild inflammation was found within the eyes. The hemorrhage found in the calvarium, when cross-sections of the head were made, are probably the result of trauma at the time of collection. However, because brain hemorrhage is one lesion seen with thiamine deficiency, in the future it would be best to avoid trauma to the head to allow better evaluation of the brain.

In the sections of gills (block #2), the lymphoid cell populations were quite variable. Some of this variation is an unavoidable result of sectioning a gill arch at different levels because the lymphoid cell population varies in density at different levels in the gill arch;

therefore, this data is subjective and to be interpreted with some caution. While difficult to acess, the lymphoid cell numbers do vary markedly and this data should be carefully compared via computer analysis with eventual survival data and other parameters which may correlate to the overall fish health. This lymphoid tissue would by its location be comparable to the bronchial-associated lymphoid tissue (BALT) of the mammalian lung. The BALT is the collection of unstimulated lymphocytes in the peribronchial tissue which respond to inhaled antigens by multiplying the producing colones of memory T cells, and B cells which produce specific antibodies against that antigen. Lymphoid cell increases noted in many of these fish gills are a non-specific indicator of exposure to antigens in the water.

The proliferative lesion of the gill epithelium suffers the same problems of interpretation that the gill lymphoid lesion did, e.g. that of the plane of section. Even the most severe (+++) lesion noted is relatively mild in degree. This lesion was not associated with histological evidence of bacterial disease and is a response to undefined damage to the gill epithelium with resultant reparative proliferation. This lesion could be compared to the proliferation of type II alveolar lining cells of the mammalian lung in response to damage, and is compatible with a very mild form of

the nutritional gill disease described in fish. The various parasites of the gills did not appear to be causing significant injury to the gill itself. Their importance may be as a subclinical disease state which will cause heavily infested populations to be unable to handle other stresses as well as less affected populations. For example, the Carson coho may not do as well as Willard coho when exposed to some additional insult because of their higher incidence of the basophillic granular organisms. These parasites have not been specifically identified, but work is continuing in this area.

A rare fish had greatly distended blood filled capillaries in occasional secondary lamellae of the gills. This lesion has been termed “hemorrhagic gill disease”, and later “toxic gill disease” by investigators who have produced the lesion with DDT and aflatoxins.

In the sections of liver and kidney (block #3), the incidence of histologically detectable kidney disease lesions was extremely low, as was the incidence of discrete granulomas. Several groups of fish had signficant although mild infiltrates of mononuclear cells. The spring chinook salmon had the highest incidence of gill lymphoid cell lesions, mononuclear cell infiltrates in the liver and granulomas in the kidney. These inflammatory lesions may be inter-related and may

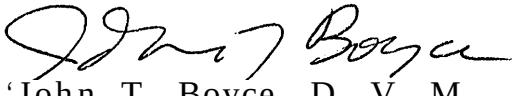
affect the overall survival of this population of fish. The focal mononuclear cell infiltrates may represent a proliferation of macrophages attempting to phagocytize material reaching the liver. This material could also be stiumlating the granuloma production in the kidney; and therefore, may represent an undetected blood-born infectious agent. While KD organisms could not be detected in these lesions, the correlation of these histological lesions with the incidence of FA positive KD infected fish.

The interpretation of gram stains is a definite problem area. The data generated is not reliable in my opinion because of technical difficulties in differentiating gram plus bacteria from other debri in the sections. I think the accuracy of this portion of the study could be improved by direct comparison of serial frozen sections of kidney tissue, staining one section with the gram stain and the adjacent section with the fluorescent antibody technique. This would need only be done on a few fish next year with some known positives to allow the pathologist to conclusively determine how much of the gram plus material is indeed coryneybacteria. This could be done at no additional charge, and the gram stains from this years fish re-evaluated. The frozen section work would also allow more precise localization of the bacteria within the tissues.

The proliferative lesion in the kidney with intracellular inclusion bodies may represent a viral infection. The morphology of the inclusions is similar to mannalial pox viruses, A portion of the lesion is being processed for electron microscopy to better define it.

SUMMARY: In this study, six groups of sixty fish each were examined histologically with particular emphasis on the eye, gill, liver and kidney. Frequent, but mild skeletal muscle myodegeneration was seen adjacent to the eye. Minimal gill epithelial lesions of the “nutritional gill disease” type were found in the gills, along with variable lymphoid hyperplasia and occasional protozoan parasites. A few fish had lesions typical of “toxic gill disease ." Rare lesions of “bacterial kidney disease” were seen in the livers and kidneys plus some nonspecific granulomas. The overall health of these fish appeared good, but the spring chinook had the highest incidence of lesions in several disease categories. The gram stain did not prove useful in detecting low numbers of gram positive bacteria in tissue sections.

Respectfully submitted,,



‘John T. Boyce, D. V. M.
Diplomate, American College
of Veterinary Pathologists

INDEX OF DATA TABLES

	EYE			GILL			LIVER				KIDNEY			GRAM		COMMENTS:	
	MYO	FAT	MISC	LYMPH	EPITH	MISC	FAT	KD	GRAN	MISC	KD	GRAN	Ca	L	K		
	CARSON COHO TOTAL INCIDENCE															BGO (GILL)	FM (LIVER)
-	73%	100%	99%	25%	39%	27%	98%	98%	97%	92%	98%	98%	100%	98%	95%	27%	92%
+	27%	0%	--	73%	61%	--	2%	0%	3%	--	0%	2%	0%	2%	5%	59%	8%
++	0%	0%	--	2%	0%	--	0%	2%	0%	--	2%	0%	0%	-	-	14%	0%
+++	0%	0%	--	0%	0%	--	0%	0%	0%	--	0%	0%	0%	-	-	0%	0%
	SPRING CHINOOK TOTAL INCIDENCE															CP (Gill)	FM (Liver)
-	88%	92%	100%	5%	83%	97%	96%	97%	98%	30%	97%	74%	100%	97%	97%	97%	30%
+	12%	7%	0%	73%	10%	--	2%	0%	0%	--	0%	23%	0%	3%	3%	3%	50%
++	0%	19%	0%	20%	7%	--	2%	3%	0%	--	0%	3%	0%	-	-	0%	15%
+++	0%	0%	0%	2%	0%	--	0%	0%	2%	--	3%	0%	0%	-	-	0%	5%
	DVORSHAK STEELHEAD TOTAL INCIDENCE															GILL	FM (LIVER)
-	37%	68%	87%	22%	13%	77%	90%	100%	100%	75%	100%	98%	100%	100%	100%		75%
+	53%	27%		65%	68%	--	0%	0%	0%	--	0%	2%	0%	0%	0%	CP 18%	22%
++	10%	3%		8%	15%	--	0%	0%	0%	--	0%	0%	0%	-	-	SP 3%	3%
+++	0%	2%		5%	4%	--	0%	0%	0%	--	0%	0%	0%	-	-		0%
	WINTHROP STEELHEAD TOTAL INCIDENCE															GILL (FD)	GILL (MP)	FM (LIVER)
-	48%	93%	100%	33%	8%	90%	97%	100%	100%	100%	100%	98%	98%			90%	90%	100%
+	48%	7%	0%	65%	78%	--	3%	0%	0%	0%	0%	2%	2%			8%	2%	0%
++	2%	0%	0%	2%	13%	--	0%	0%	0%	0%	0%	0%	0%					0%
+++	2%	0%	0%	0%	0%	--	0%	0%	0%	0%	0%	0%	0%					0%
	TUCANNON STEELHEAD TOTAL INCIDENCE																	FM (LIVER)
-	43%	100%	88%	78%	67%	97%	92%	100%	98%	82%	100%	93%	100%			12% intracranial hemorrhage*		82%
+	48%	0%	12%	22%	33%	3%	8%	0%	2%	-	0%	7%	0%			2% CD in gill		16%
++	9%	0%	0%	0%	0%	0%	0%	0%	0%	-	0%	0%	0%			2% neuts in gill		2%
+++	0%	0%	0%	0%	0%	0%	0%	0%	0%	-	0%	0%	0%					0%
	WILLARD COHO SALMON (58 total fish)															GILL		FM (LIVER)
-	60%	93%	97%	78%	55%	88%	100%	100%	97%	97%	100%	96%	100%			BGO + 5%		97%
+	36%	7%	--	22%	41%	--	0%	0%	3%	3%	0%	2%	0%			BGO ++ 5%		3%
++	4%	0%	--	0%	4%	--	0%	0%	0%	0%	0%	2%	0%					0%
+++	0%	0%	--	0%	0%		0%	0%	0%	0%	0%	0%	0%					0%

*ICH

4401-4430	6 MYO	8 EYE FAT	EYE MISC	26 GILL LYMPH	30 GILL EPITH	GILL MISC	50 LIVER FAT	52 LIVER KD	54 LIVER GRAN	LIVER MISC	60 KIDNEY KD	62 KIDNEY GRAN	64 KIDNEY Ca	70 GRAM L	72 GRAM K	COMMENTS:
4401	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	
02	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	BGO = Basophillic granular organism
03	-	-	-	-	-	BGO++	-	-	-	-	-	-	-	-	-	
04	-	-	-	-	-	BGO++	-	-	-	-	-	-	-	-	-	
05	+	-	-	-	-	BGO++	-	-	-	-	-	-	-	-	-	
06	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
07	+	-	-	-	+	FB	-	-	-	-	-	-	**	-	-	FB=Filamentous bacteria, **See below
08	+	-	-	-	+	-	-	-	-	FM	-	-	-	-	-	FM = Focal mononuclear cell infiltrate
09	-	-	-	+	+	BGO	-	++	-	-	++	-	-	+	+	
4410	-	-	-	+	+	BGO++	-	-	-	-	-	-	-	-	-	
11	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	Rare G+ bacteria in kidney
12	+	-	-	+	+	BGO++	-	-	-	-	-	-	-	-	-	
13	+	-	-	-	-	BGO	-	-	-	-	-	-	-	-	-	
14	+	-	-	-	+	-	-	-	-	-	-	-	*	-	-	*G+ debri in tubles
15	+	-	-	+	+	BGO++	-	-	-	-	-	-	-	-	-	
16	-	-	-	-	-	BGO	+	-	-	-	-	-	-	-	-	
17	+	-	-	+	+	BGO	-	-	-	FM	-	-	-	-	-	
18	-	-	-	-	-	BGO	-	-	+	-	-	-	-	-	-	
19	+	-	-	+	+	BGO	-	-	-	-	-	-	-	-	+	
4420	-	-	-	-	-	BGO	-	-	-	-	-	-	*	-	-	*G+ debri in tubles
21	+	-	-	-	-	BGO	-	-	+	-	-	-	-	-	-	
22	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
23	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
24	+	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
25	-	-	-	+	+	BGO++	-	-	-	-	-	-	-	-	-	
26	-	-	-	+	+	BGO	-	-	-	-	-	+	-	-	-	
27	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
28	-	-	-	+	+	BGO++	-	-	-	-	-	-	-	-	-	
29	-	-	SI/σ	+	+	BGO	-	-	-	-	-	-	-	-	-	SI = minimal subacute iridocyclitis
4430	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
SUBTOTALS:																
-	18	30	29	12	8	5	29	29	28	28	29	29	27	28	26	
+	12	0	-	18	22	16	1	0	2	2	0	1	-	1	3	
++	0	0	-	0	0	8	0	1	0	0	1	0	-	-	-	
+++	0	0	-	0	0	0	0	0	0	0	0	0	-	-	-	

**G+debri in tubles 66

4431-4460	EYE			GILL			LIVER				KIDNEY			GRAM		COMMENTS:
	MYO	FAT	MISC	LYMPH	EPITH	MISC	FAT	KD GRAN		MISC	KD	GRAN	Ca	L	K	
4431	-	-	-	-	+	BGO	-	-	-	-	-	-	-	-	-	
32	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
33	+	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
34	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
35	-	-	-	+	-	BGO	-	-	-	-	-	-	-	-	-	
36	-	-	-	-	-	BGO	-	-	-	-	-	-	-	-	-	
37	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
38	-	-	-	+	+	BGO	-	-	-	-	-	++	-	-	-	
39	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
4440	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
42	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	**	Possible G+ bacteria in tubular lumen
43	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
44	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
45	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
46	+	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	
47	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
48	-	-	-	+	+	BGO	-	-	-	-	-	-	-	-	-	
49	-	-	CI	+	-	BGO	-	-	-	-	-	-	-	-	-	CI = choroid gland inflammation
4450	-	-	-	+	-	BGO	-	-	-	FM	-	-	-	-	-	
51	-	-	-	+	-	BGO	-	-	-	-	-	-	-	-	-	
52	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
53	-	-	-	+	-	BGO	-	-	-	FM	-	-	-	-	-	
54	-	-	-	+	-	BGO	-	-	-	-	-	-	-	-	-	
55	-	-	-	+	-	BGO	-	-	-	-	-	-	-	-	-	
56	-	-	-	+	-	BGO	-	-	-	-	-	-	-	-	-	
57	-	-	-	+	-	BGO	-	-	-	-	-	-	-	-	-	
58	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
59	-	-	-	+	-	BGO	-	-	-	-	-	-	-	-	-	
4460	No fish found															
FINAL TOTALS:																
-	43	59	58	15	23	15	58	58	59	54	58	57	55	58	56	
+	16	0	-	43	36	BGO35	1	0	2	5	0	1	-	1	3	
++	0	0	-	1	0	BGO 8	0	1	0	0	1	1	-	-	-	
				0	0		0	0	0	0	0	0	-	-	-	

1001-1030	EYE			GILL			LIVER				KIDNEY			GRAM		COMMENTS:
	MYO	FAT	MISC	LYMPH	EPITH	MISC	FAT	KD GRAN		MISC	KD	GRAN	Ca	L	K	
1001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
02	-	-	-	+	-	-	-	-	-	FM++	-	-	+	-	-	FM = Focal mononnclears
03	-	-	-	+	-	-	-	-	+++	-	-	+	-	-	-	Granulomatous hepatitis
04	+	++	-	++	-	-	-	-	-	FM	-	-	+	-	-	
05	0	0	0	+	-	-	-	-	-	-	-	-	+	-	-	
06	-	+	-	++	+	-	-	-	-	FM+++	-	-	-	-	-	Hepatocytic necrosis
07	-	+	-	+	-	-	-	-	-	FM++	-	-	+	-	-	
08	-	-	-	++	++	-	-	-	-	-	-	-	-	-	-	
09	-	+	-	++	-	-	-	-	-	FM	-	-	-	-	-	
1010	-	+	-	++	++	-	-	-	-	-	-	-	-	-	-	
11	-	-	-	+	+	-	-	-	-	FM	-	-	+	-	-	
12	-	-	-	+	-	-	-	-	-	FM	-	-	-	-	-	
13	-	-	-	++	+	-	-	-	-	-	-	++	+	-	-	
14	-	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	
15	-	-	-	+	-	-	-	-	-	FM	-	+	-	-	-	
16	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
17	-	-	-	++	-	-	-	-	-	FM	-	-	++	-	-	
18	-	-	-	+	-	-	-	-	-	FM++	-	-	+	-	-	
19	-	-	-	+	-	-	-	-	-	FM++	-	-	-	-	-	
1020	-	-	-	-	-	-	-	-	-	FM	-	-	-	-	-	
21	+	-	-	+	-	-	-	-	-	FM	-	-	+	-	-	
22	-	-	-	++	+	-	-	-	-	FM	-	-	+	-	-	
23	-	-	-	+	-	-	-	-	-	-	-	+	+	-	-	
24	-	-	-	++	-	-	+	-	-	FM++	-	-	+	-	-	
25	-	-	-	-	-	-	-	-	-	-	-	-	++	-	-	
26	+	-	-	+++	++	-	-	-	-	FM++	-	++	-	-	-	
27	-	-	-	+	-	-	-	-	-	FM	-	+	-	-	-	
28	-	-	-	+	-	-	-	-	-	FM	-	+	-	-	-	
29	-	-	-	+	-	-		-	-	FM	-	+	-	-	-	
1030												+	+++	-	-	
SUBTO																
-	27	25	30	3	22	30	29	30	29	10	30	21	16	30	30	
+	3	4	0	17	5	0	1	[illegible]			[illegible]			-	-	
++	0	1	0	9	3	0	0	0	0	6	0	2	2	-	-	
	0	0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-	-	

4031-4060	EYE MYO	EYE FAT	EYE MISC	GILL LYMPH	GILL EPITH	GILL MISC	LIVER FAT	LIVER KD	LIVER GRAN	LIVER MISC	KIDNEY KD	KIDNEY GRAN	KIDNEY Ca	GRAM L	GRAM K	COMMENTS:
4031	-	-	-	+	-	-	-	-	-	FM	-	+	-	-	-	
32	+	-	-	+	-	-	-	-	-	FM	-	+	++	-	-	
33	-	-	-	+	-	-	-	-	-	FM	-	+	-	-	-	
34	-	-	-	++	-	-	-	-	-	FM	-	+	-	-	-	
35	-	-	-	+	-	-	-	++	-	FM	+++	-	-	+	+	
36	-	-	-	+	-	-	-	-	-	FM+++	-	-	-	-	-	
37	-	-	-	+	-	-	-	-	-	FM	-	+	+	-	-	
38	-	-	-	+	-	-	-	-	-	-	-	-	++	-	-	
39	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
4040	-	-	-	++	++	CP	++	++	-	FM	+++	-	-	+	+	CP = Cil
41	+	-	-	+	-	-	-	-	-	FM	-	-	-	-	-	
42	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
43	+	-	-	+	-	-	-	-	-	FM	-	+	-	-	-	
44	-	-	-	+	-	-	-	-	-	FM++	-	-	+	-	-	
45	-	-	-	+	-	-	-	-	-	FM	-	-	+	-	-	
46	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	
47	-	-	-	+	-	CP	-	-	-	FM	-	-	-	-	-	
48	-	-	-	++	-	-	-	-	-	FM+++	-	-	++	-	-	
49	-	-	-	+	-	-	-	-	-	FM	-	-	-	-	-	
4050	-	-	-	+	-	-	-	-	-	FM	-	-	-	-	-	
51	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
52	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	
53	-	-	-	+	-	-	-	-	-	FM	-	-	-	-	-	
54	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	
55	-	-	-	+	+	-	-	-	-	FM	-	+	+	-	-	
56	-	-	-	+	-	-	-	-	-	-	-	-	+++	-	-	
57	-	-	-	+	-	-	-	-	-	FM	-	-	-	-	-	
58	-	-	-	+	-	-	-	-	-	FM++	-	-	-	-	-	
59	-	-	-	+	-	-	-	-	-	FM++	-	-	+	-	-	
4060	-	-	-	+	-	-	-	-	-	FM	-	-	-	-	-	
FINAL TOTALS:																
-	53	55	60	3	50	58	58	59	59	18	58	39	25	58	58	
+	7	4	0	44	6	CP2	1	0	0	30	0	18	28	2	2	
++	0	1	0	13	4	0	1	2	0	9	0	2	5	0	0	
+++	0	0	0	1	0	0	0	0	1	3	2	1	2	0	0	

4061-4090	EYE MYO	EYE FAT	EYE MISC	GILL LYMPH	GILL EPITH	GILL MISC	LIVER FAT	LIVER KD	LIVER GRAN	LIVER MISC	KIDNEY KD	KIDNEY GRAN	KIDNEY Ca	GRAM L	GRAM K	COMMENTS:
4061	+	-	-	+	+	-	+	-	-	-	-	-	-	-	-	
62	-	+	-	+	+	-	+	-	-	FM	-	-	-	-	-	
63	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
64	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
65	+	-	-	+	++	-	-	-	-	-	-	-	-	-	-	
66	+	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	
67	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	
68	++	+++	**	-	-	-	-	-	-	-	-	-	-	-	-	**Chronic active opthalmitis
69	-	-	-	+	+	CP	-	-	-	-	-	-	-	-	-	CP = Ciliated protozoan
4070	-	-	-	+	++	-	-	-	-	FM	-	-	-	-	-	
71	+	++	-	+	+	-	-	-	-	-	-	-	-	-	-	
72	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	
73	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	
74	+	-	-	-	+	**	-	-	-	FM	-	-	-	-	-	**Questionable protozoan parasite
75	+	+	-	-	+	CP	-	-	-	-	-	-	-	-	-	
76	+	+	-	-	++	-	-	-	-	FM	-	-	-	-	-	
77	+	-	-	-	+	CP	-	-	-	-	-	-	-	-	-	
78	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	
79	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
4080	-	-	-	+	++	-	-	-	-	-	-	-	-	-	-	
81	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	
82	++	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
83	+	-	-	+	+	-	+	-	-	-	-	-	-	-	-	
84	+	-	-	+	+	-	+	-	-	-	-	-	-	-	-	
85	-	-	-	+	+	**	+	-	-	-	-	-	-	-	-	**Solitary basophillic mass in SL
86	-	-	-	++	++	CP	-	-	-	-	-	-	-	-	-	
87	-	+	FM	+	+	-	-	-	-	FM	-	-	-	-	-	Focal mononuclears in optic nerve
88	+	-	-	+	+	-	-	-	-	FM	-	-	-	-	-	
89	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	
4090	-	-	-	+	+++	-	-	-	-	FM	-	-	-	-	+	Gram+ bacteria (?) in kidney
SUBTOTALS:																
-	15	21	28	10	3	24	24	30	30	22	30	30	30	30	30	
+	13	7	-	19	21	CP4	6	0	0	FM8	0	0	0	0	0	
++	2	1	-	1	-	0	-	-	-	-	0	-	0	-	-	
+++	0	1	-	0	1	0	0	0	0	0	0	0	0	-	-	

4091-4020	EYE			GILL			LIVER				KIDNEY			GRAM		COMMENTS:
	MYO	FAT	MISC	LYMPH	EPITH	MISC	FAT	KD	GRAN	MISC	KD	GRAN	Ca	L	K	
4091	+				+					-		-	-	-	-	
92	+	+	-	+	+	CP	-					-	-	-	-	CP = Ciliated protozoan
93	+	+		-	+				-	-		-	-	-	-	
94	+++		I	+	+	I	I	I	-	-	I	-	-	-	-	
95	+	-	-	++	++	-	-	-	-	-	-	-	-	-	-	
96	+	-	-	+	++	-	-	-	-	-	-	-	-	-	-	
97	+	-	- I	+	+	-	-	-	-	-	-	-	I	-	-	
98	++	-	-	+	+	CP	-	-	-	-	-	-	-	-	-	
99	-	+	**	+	+	CP	-	-	-	-	-	-	I	-	-	**Cornea-scleral inflammation
4100	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
01	+	+	-	+	-	-	-	-	-	FM	-	-	-	-	-	
02	++	++	-	++	++	CP/SP	-	-	-	-	-	-	-	-	-	SP = sporozoan protozoan parasite
03	+	+	**	+++	++		-					-	-		-	**Neuritis (optis nerve)
04	-	-	-	++					-	FM	-	-	-	-	-	
05	+	+	**	+	-	-	-	-	-	-	-	-	-	-	-	**neuritis (optic nerve)
06	-	-		++	+		-		-	FM++	-	-	-	-	-	
07	-	-	-	+++	++							+	-	-	-	
08	-	-	-	+	-	-	-	-	FM	-	-	-		-	-	
09	-	-	-	+++	+	SP	-	-	-	-	-	-	-	-		Sporozoan protozoan parasite
4110	+	-								FM	-	-	-		-	
11	++	-	IHC	+	+							-		-	-	IHC = Brain hemorrhage
12	+	-		+	+	CP	-			FM++	-	-	-	-	-	
13	+	-		+	+		-	-	-	-	-	-	-	-	-	
14	+	-		+	+	CP	-		-			-		-	-	
15	+	-		+	+				-			-		-	-	
16	+	-	-	+	+				-			-	-	-	-	
17	+	-	-	+	+				-	FM	-	-	-	-	-	
18	+	-	-	+	+					-	-	-	-	-	-	
19	+	+	FM	+	+	CP	-	-	-	-	-	-	-	-	-	FM-Focal mono's in brain
4120	-	+	-	+	+						-	-	-	-	-	
FINAL TOTALS:																
	22	41	54	13	8	46	54	60	60	45	60	59	60	60	60	
+	32	16	-	20	20	CP7	0	0	0	FM13	0	1	0	0	0	
++	6	2	-	4	4	00		0	0	FM 2	0	0	0	-	-	
+++	0	1	-	3	1	0	0	0	0	0	0	0	0	-	-	

4501-4530	EYE			GILL			LIVER				KIDNEY			GRAM		COMMENTS:
	MYO	FAT	MISC	LYMPH	EPITH	MISC	FAT	KD GRAN		MISC	KD	GRAN	Ca	L	K	
4501	+	-	-	+	++	-	-	-	-	-	-	-	-			
02	-	-	-	-	-	FD	-	-	-	-	-	-	+			Fd = Focal capillary dilation in S.L.
03	-	-	-	+	+	-	-	-	-	-	-	-	-			
04	-	-	-	++	++	FD	-	-	-	-	-	-	-			
05	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	
06	-	-	-	+	+	-	-	-	-	-	-	-	-			
07	++	*	-	-	+	-	-	-	-	-	-	-	-			*Focal hemorrhage in fat
08	-	-	-	-	+	-	-	-	-	-	-	-	-			
09	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
4510	-	-	-	-	+	FD	-	-	-	-	-	-	-			
11	-	-	-	-	+	-	-	-	-	-	-	-	-			
12	+	-	-	+	+	-	-	-	-	-	-	-	-			
13	-	-	-	-	++	-	-	-	-	-	-	-	-			
14	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
15	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
16	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	
17	+	-	-	+	++	-	-	-	-	-	-	-	-			
18	+	-	-	-	+	-	-	-	-	-	-	-	-			
19	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	
4520	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	
21	-	-	-	-	+	-	-	-	-	-	-	-	-			
22	+	-	-	-	+	-	-	-	-	-	-	-	-			
23	-	-	-	+	+	-	-	-	-	-	-	-	-			
24	+	+	-	-	+	-	-	-	-	-	-	-	-			
25	-	-	-	-	++	-	-	-	-	-	-	-	-			
26	-	-	-	+	+	-	-	-	-	-	-	-	-			
27	+	-	-	+	++	-	-	-	-	-	-	-	-			
28	+	-	-	+	+	-	-	-	-	-	-	-	-			
29	-	-	-	+	+	-	-	-	-	-	-	-	*			*De-ri in excretory duct
4530	-	-	-	+	+	FD	+	-	-	-	-	-	-			
SUBTOTALS:																
-	19	27	30	14	2	26	29	30	30	30	30	29	28	-	-	
+	10	2	0	15	22	FD4	1	0	0	0	0	1	1	-	-	
++	1	0	0	1	6	0	0	0	0	0	0	0	0	-	-	
+++	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	

4531-4560	EYE			GILL			LIVER				KIDNEY			GRAM	COMMENTS:
	MYO	FAT	MISC	LYMPH	EPITH	MISC	FAT	KD	GRAN	MISC	KD	GRAN	Ca	L K	
4531	+	-	-	+	+	-	-	-	-	-	-	-	-		
32	+	-	-	-	+	-	-	-	-	-	-	-	-		
33	-	-	-	+	++	-	-	-	-	-	-	-	-		
34	+	-	-	+	+	-	-	-	-	-	-	-	-		
35	+	-	-	+	+	-	-	-	-	-	-	-	-		
36	+	-	-	+	+	-	-	-	-	-	-	-	-		
37	+	-	-	+	+	-	+	-	-	-	-	-	-		
38	-	-	-	-	-	-	-	-	-	-	-	-	-		
39	+	-	-	+	-	-	-	-	-	-	-	-	-		
4540	+	-	-	+	+	-	-	-	-	-	-	-	-		
41	-	+	-	+	+	-	-	-	-	-	-	-	-		
42	-	-	-	+	+	-	-	-	-	-	-	-	-		
43	+++*	-	-	+	+	-	-	-	-	-	-	-	-		*Marked muscle regeneration
44	+	-	-	+	+	-	-	-	-	-	-	-	-		
45	+	-	-	+	+	-	-	-	-	-	-	-	-		
46	+	-	-	-	+	-	-	-	-	-	-	-	-		
47	-	-	-	+	+	-	-	-	-	-	-	-	-		
48	-	-	-	-	-	-	-	-	-	-	-	-	-		
49	+	-	-	+	+	MP*	-	-	-	-	-	-	-		*Microsporidian protozoan parasite
4550	+	-	-	+	+	FD	-	-	-	-	-	-	-		FD = Focal capillary dilatation
51	+	-	-	+	++	-	-	-	-	-	-	-	-		
52	+	-	-	-	+	-	-	-	-	-	-	-	-		
53	-	-	-	-	+	-	-	-	-	-	-	-	-		
54	+	-	-	+	+	-	-	-	-	-	-	-	-		
55	+	-	-	+	+	-	-	-	-	-	-	-	-		
56	-	-	-	+	+	-	-	-	-	-	-	-	-		
57	-	-	-	+	+	-	-	-	-	-	-	-	-		
58	+	-	-	+	+	-	-	-	-	-	-	-	-		
59	+	-	-	+	+	-	-	-	-	-	-	-	-		
4560	-	-	-	+	+	-	-	-	-	-	-	-	-		
FINAL TOTALS:															
-	29	56	60	20	5	54	58	60	60	60	60	59	60		
+	29	3	0	39	47	FD5	2	0	0	0	0	1	0		
++	1	-	-	1	8	-	-	-	-	-	-	-	-		
+++	1	-	-	-	-	-	-	-	-	-	-	-	-		

4601-4630	EYE MYO	EYE FAT	EYE MISC	GILL LYMPH	GILL EPITH	GILL MISC	LIVER FAT	LIVER KD	LIVER GRAN	LIVER MISC	KIDNEY KD	KIDNEY GRAN	KIDNEY Ca	GRAM L K	COMMENTS:
4601	-	-	MH	-	+	-	-	-	-	EM	-	-	-		MH = Meningeal hemorrhage (ICH)
02	-	-	MH	-	-	-	-	-	-	-	-	-	-		
03	+	-	-	-	-	CD	-	-	-	-	-	-	-		CD = Capillary dilitation in S. L.
04	+	-	-	-	-	-	-	-	-	-	-	-	-		
05	-	-	ICH	-	-	-	-	-	-	-	-	+	-		ICH-Cerebral hemorrhage
06	+	-	-	-	+	-	-	-	-	-	-	-	-		
07	+	-	-	-	-	-	-	-	-	-	-	-	-		
08	+	-	-	+	+	-	-	-	-	-	-	-	-		
09	+	-	-	-	+	-	+	-	-	-	-	-	-		
4610	+	-	-	-	-	-	-	-	-	-	-	-	-		
11	+	-	-	-	-	-	-	-	-	-	-	-	-		
12	++	-	-	-	-	-	+	-	-	-	-	-	-		
13	+	-	-	-	-	-	+	-	-	-	-	-	-		
14	-	-	-	+	+	-	-	-	-	-	-	-	-		
15	-	-	-	+	+	-	+	-	-	-	-	-	-		
16	+	-	-	-	-	-	+	-	-	-	-	-	-		
17	-	-	-	+	+	-	-	-	-	EM	-	-	-		
18	+	-	-	-	+	-	-	-	-	-	-	-	-		
19	+	-	-	-	-	-	-	-	-	EM	-	-	-		
4620	+	-	-	+	+	-	-	-	-	EM	-	-	-		
21	+	-	-	-	-	-	-	-	-	EM	-	+	-		
22	+	-	-	-	-	-	-	-	-	-	-	-	-		
23	-	-	-	+	+	**	-	-	-	-	-	-	-		**Neutrophiles in secondary lamellae
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	+	-	-	-	-	-	-	-	-	-	-	-	-		
26	-	-	ICH	-	-	-	-	-	-	EM	-	-	-		
27	++	-	ICH	-	-	-	-	-	-	-	-	-	-		
28	++	-	-	-	-	-	-	-	-	-	-	-	-		
29	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4630	-		ICH	-	-	-	-	-	-	-	-	-	-	-	-
SUBTOTALS:															
-	10	30	24	24	20	28	25	30	30	25	30	28	30	- -	
+	6	0	ICH6	6	10	0	5	0	0	EM5	0	2	0	- -	
++	4	0	0	0	0	0	0	0	0	0	0	0	0	- -	
+++	0	0	0	0	0	0	0	0	0	0	0	0	0	- -	

4631-4660	EYE			GILL			LIVER				KIDNEY			GRAM	
	MY0	FAT	MISC	LYMPH	EPITH	MISC	FAT	KD	GRAN	MISC	KD	GRAN	Ca 1,	K	COMMENTS:
4631	+	-	-	-	+					FM					FM = Focal mononuclears
32	-		-	-I-	+							**	-		**Foci of neuronhiles
33	-	-	-	+	+										
34	-	-	-	-	-	-				-I-		-	-		
35		-	-		+	-	-			-I-		-	-		
36		-	-	-	-	-	-								
37	+	-		+											
38	+														
39	-	-		-I-	-	-				-I-		-	-		
4640		-	-	-	-	-	-				-I-		-	-I	
41	+	-	-	+						-I-		+		-I	
42	+	-		-							-				
43	-		CH	+	+	-					-				ICH = cerebral hcmorrhaee
44	-			+						-	-				
45	-			-			-	-	-	-	-	+++	-	-	
46	-		**	-	+		-	-	+	-	-	-	-	-	**Segmental vasculitis
47	+	-		-I-	+		-				-			-	
48	-	-	-	+	+		-	-	-	-	-	-	-		
49	+	-	-	-	-		-	-	-			-I--		-I	
4650	+	-	-	-	-	-	-	-	-	FM	-	-		-	
51	+	-	-	-	-	-	-	-	-	-	-	-	-		
52															
53	+	-	-	-	+		-	-	-	FM	-	-	-		
54	+		-	-I-	+		-	-	-	FM++	-				
55	++	-	-	-			-	-	-	FM	-	-	-		
56	+	-	-	-	-	-	-	-	-	-I-		-	-		
57	-	-	-	-	-	-	-	-	-	-	-	-	-		
58	+	-		-						FM	-	-	-		
59	-	-	-	-	-	-	-	-	-	-	-	-	-		
4660					+										
FINAL TOTALS:															
	26	60	52	47	40	58	55	60	59	29	60	55	60	3	3
+	29	0	ICH7	13	20	-	5	0	1	FM10	0	3	0	0	C
++	1	0	0	0	0	-	0	0	0	FM1	0	0	00	0	
+++	0	0	0	0	0	-	0	0	0	0	00		0	0	

4351-4670	EYE			GILL			LIVER				KIDNEY			GRAM	
	MYO	FAT	MISC	LYMPH	EPITH	MISC	FAT	KD	GRAN	MISC	KD	GRAN	Ca	L	K	COMMENTS:
4351	-	-	-	-	-	BGO	-	-	-	FM	-	-	-			BGO = Basophillic granular organism
52	-	-	-	-	-	-	-	-	-	-	-	-	-			
53	+	-	-	-	+	-	-	-	-	-	-	-	-			
54	-	-	-	-	-	-	-	-	-	-	-	-	-			
55	+	-	-	-	+	-	-	-	-	-	-	-	-			
56	-	-	-	-	-	-	-	-	-	-	-	-	-			
57	+	-	-	-	-	-	-	-	-	-	-	-	-			
58	-	-	-	-	-	-	-	-	-	FM	-	-	-			
59	+	-	-	-	-	-	-	-	-	-	-	-	-			
4360	-	-	-	+	+	-	-	-	-	-	-	-	-			
61	-	-	-	-	+	-	-	-	-	-	-	-	-			
62	-	-	ICH	-	-	-	-	-	-	-	-	-	-			ICH = Intracranical hemorrhage
63	-	-	-	-	+	-	-	-	-	-	-	-	-			
64	-	-	-	-	-	-	-	-	-	-	-	-	-			
65	+	-	-	-	-	-	-	-	-	FM	-	-	-			
66	+	-	-	-	-	-	-	-	-	-	-	-	-			
67	-	-	-	+	-	-	-	-	-	-	-	-	-			
68	-	-	-	-	-	-	-	-	-	-	-	-	-			
69	-	-	-	-	-	BGO++	-	-	+	-	-	-	-			
4370	+	-	-	-	-	-	-	-	-	-	-	-	-			
4661	-	-	-	-	+	-	-	-	-	-	-	-	-			
62	+	+	-	-	-	BGO	-	-	-	-	-	-	-			BGO = Basophillic granular organism
63	-	-	-	-	-	-	-	-	-	-	-	-	-			
64	+	-	-	-	+	-	-	-	-	-	-	-	-			
65	-	-	-	-	-	-	-	-	-	-	-	-	-			
66	++	+	-	-	-	-	-	-	-	-	-	-	-			
67	-	-	-	+	+	-	-	-	-	-	-	-	-			(Slide mislabeled as 4067)
68	+	-	-	-	-	-	-	-	-	-	-	-	-			
69	-	-	-	-	+	BGO+++	-	-	-	-	-	-	-			
4670	-	+	-	-	+	-	-	-	-	-	-	-	-			
SUBTOTALS:																
-	19	27	29	27	20	26	30	30	29	27	30	30	30	-	-	
+	10	3	ICH-	3	0	2	0	0	1	FM3	0	0	0			
++	1	0	0	0	0	1	0	0	0	0	0	0	0			
+++	0	0	0	0	0	1	0	0	0	0	0	0	0			

4671 4700	EYE MYO	EYE FAT	EYE MISC	GILL LYMPH	GILL EPITH	GILL MISC	LIVER FAT	LIVER KD	LIVER GRAN	LIVER MISC	KIDNEY KD	KIDNEY GRAN	KIDNEY Ca	GRAM L K	COMMENTS:
4671	-	-	-	-	++	-	-	-	-	-	-	-	-		
72	-	-	-	+	+	-	-	-	-	-	-	-	-		
73	-	-	-	+	++	-	-	-	-	-	-	-	-		
74	-	-	-	-	-	-	-	-	-	-	-	++	-		
75	+	-	-	-	-	-	-	-	-	-	-	-	-		
76	+	-	-	+	+	-	-	-	-	FN	-	-	-		FN = Focal necrosis
77	+	-	-	+	+	-	-	-	-	-	-	-	-		
78	+	-	-	+	+	-	-	-	-	-	-	-	-		
79	++	-	-	-	+	-	-	-	-	-	-	-	-		
4680	+	-	-	+	+	-	0	0	0	0	-	-	-		(No liver imbedded)
81	-	+	-	-	+	-	-	-	-	-	-	+	-		
82	-	-	-	+	+	-	-	-	-	-	-	-	-		
83	-	-	-	-	-	-	-	-	-	-	-	-	-		
84	No fish found														
85	+	-	-	-	+	-	-	-	-	-	-	-	-		
86	+	-	-	-	-	CG	-	-	-	-	-	-	-		CG = Congested gills
87	-	-	-	+	+	BGO++	-	-	-	-	-	-	-		
88	-	-	-	-	+	-	-	-	-	-	-	-	-		
89	+	-	-	-	+	BGO	-	-	+	-	-	-	-		
4690	-	-	-	-	-	-	-	-	-	FM	-	-	-		FM = Focal mononuclear cell intiltrat
91	-	-	-	+	+	-	-	-	-	-	-	-	-		
92	-	-	CC	-	-	-	-	-	-	-	-	-	-		CC = Chronic conjunctivitis
93	-	-	-	-	-	-	-	-	-	-	-	-	-		
94	+	-	-	-	-	-	-	-	-	-	-	-	-		
95	-	-	-	-	-	-	-	-	-	-	-	-	-		
96	-	-	-	+	+	-	-	-	-	-	-	-	-		
97	+	-	-	-	-	-	-	-	-	-	-	-	-		
98	No fish found														
99	-	-	-	-	-	-	-	-	-	-	-	-	-		
4700	+	-	-				-	-	-	-	-	-	-		
FINAL TOTALS:															
-	35	54	46	45	32	51	57	57	55	52	58	26	58		
+	21	4	0	13	24	3	0	0	2	2	0	1	0		
++	2	0	0	0	2	3	0	0	0	0	0		0		
		0	0	0	0	1		0	0	0	0	0	0		

APPENDIX c

INDIRECT FLUORESCNET ANTIBODY TECHNIQUE (IFAT) FOR BACTERIAL KIDSEY DISEASE (BKD)

APPENDIX C. Indirect fluorescent antibody technique (IFAT) for bacterial kidney disease (BKD).

A. REAGENTS.

1. Rabbit anti-BKD serum. Biomed Research Laboratories (Seattle). Titer of 1:512. Hold at -20°C. Store in 0.1 or 0.2 ml aliquots. Dilute aliquots 1:50 in phosphate buffered saline as needed. Holds for 2 to 4 weeks at 2-4°C.

2. Goat anti-rabbit fluorescein isothiocyanate (FITC). Difco Laboratories (Detroit). (goat anti-rabbit serum; fluorescein conjugated 'IgG fraction). Rehydrate as per instructions. Freeze in 0.1 ml aliquots until needed.

3. Rhodamine counterstain. Difco Laboratories. Rehydrate as per instructions. Freeze in 0.1 ml aliquots until needed.

4. Carbonate-bicarbonate buffer (C-BB).

26.5 g Na_2CO_3 with distilled water to 500 ml final volume. Refrigerate until needed.

21.0 g NaHC03 with distilled water to 500 ml final volume. Refrigerate until needed.

For fresh C-B buffer, mix 1 part Na2C03 solution with 4 parts NaHC03.

5. Phosphate buffered saline (Kawamura's) (PBS).

8 g NaCL; 0.2 g KCl; 1.15 g anhydrous Na_2HPO_4; and 0.2 g KH_2PO_4 dissolved in 1,000 ml distilled water (pH is 7.2-7.4). Prepare fresh, or as a lo-fold concentrate without NaCl. Store concentrate at 4OC until needed; dilute and add NaCl.

6. Fluorescent antibody (FA) mounting fluid. pH 9. Difco Laboratories or: 0.5 M carbonate buffer (C-BB) (pH 9.5).....1 volume; plus 9 volumes reagent grade glycerine. Mix thoroughly.

7. Low fluorescence immersion oil.

8. Rabbit antisera for furunculosis or enteric redmouth (ERM) disease as desired for control tests. Dilute approximately 1:50 with PBS.

9. Normal rabbit antiserum (RAS). Dilute 1:50 with PBS for control tests.

B. PROCEDURE FOR PREPARING IFAT-BKD SLIDE MATERIAL.

1. Mix 0.1 ml of goat FITC with 0.1 ml of rhodamine counterstain and dilute with 4.8 ml PBS. This is a 1:20 dilution.

2. The bottom row of the 12 well multi-spot control slide is used to replicate the following controls on the top row:

(a) goat FITC conjugate only; (b) normal RAS; (c) Furunculosis RAS; (d) ERM-RAS; (e) BKD-RAS; (f) BKD-RAS.

3. Layer a plastic box with wet sponges to provide a moist chamber, place a wire rack over the sponges, and place the slides on the rack.

4. Place one drop of dilute BKD-RU on each well of the test groups and the appropriate control wells.

5. Place drops of the appropriate antisera on the remaining control wells (except the FITC conjugate only).

6. Seal the moist chamber and incubate the slides for 15 to 30 minutes at room temperature.

7. Flush the slides with PBS and transfer to slide staining dishes filled with PBS for 10 minutes.

8. Remove the slides and dry with a portable (cosmetic) hair dryer (with no heat).

9. One drop of the goat FITC counterstain conjugate is placed on each well of both test and control slides, and the slides are returned to the moist chamber for 15 to 30 minutes.

10. Rinse the slides with freshly prepared C-BB and transfer to slide staining dishes filled with C-BB for 10 minutes.

11. The slides are blower dried again, and one drop of FA mountant is placed on each well. Cover slips of 22 x 22 mm and 22 x 50 mm are used to completely cover the 25 x 75 mm slides. The slips are pressed down, butted, and small drops of cosmetic nail polish are used on corners to prevent the cover slips from moving or used to seal the entire edge. The lacquer is allowed to dry, and the slides are stored at 4°C until they are read.

C. EXAMINATION OF IFAT-BKD SLIDE MATERIAL.

Generally, slides are read within 24 to 48 hours of mounting, although no problems have been noted with BKD fluorescence for test storage periods of mounted material in excess of 30 days at 4°C, provided that the entire slide is sealed with lacquer.

The microscope used for reading the IFAT-BKD slides is a Zeiss with an pie-illuminator (UV), 12.5x oculars, and a 100x planachromatic lens with a numerical aperature of 1.25. Zeiss low fluorescence immersion oil is used.

The control slide is scanned first to ensure that all of the procedures went normally, and the intensity of fluorescence in the BKD control is subjectively noted. Generally, there has never been a problem with the control slides using this procedure.

We examined approximately 250 microscope fields (mfs) of anterior and posterior kidney in the slides from the first groups of fish. However, we noted that if any BKD organisms were going to be encountered, it would probably be within the first 100 mfs. Therefore, we felt that we could safely reduce the field scanning to 150 mfs, and all slides are now examined in this manner. Currently, we are also counting the number of BKD organisms/l50 mfs's in an attempt to determine the relative intensity of infection.

The only serious BKD-RAS cross-reactivity that was occasionally encountered was a bacillus that was easily recognized by its large size (2 to 3 times that of BKD) andv perfect shape. perfect shape.

APPENDIX D

ANALYTICAL PROCEDURE FOR MEASURING GILL Na+-K+ XTPASE ACTIVITY

APPENDIX D. Analytical Procedure for Measuring Gill Na+-K+ ATPase activity.

ENZYME PREPARATION

For ATPase determinations, samples of gill filaments were thawed and briefly homogenized in a conical glass homogenizer (7 to 12 strokes). The homogenizer was reused with 1 ml SEI which was then added to the original homogenate. The combined value (2 ml total) was placed in ice water in a centrifuge tube. To each 2 ml volume of homogenate was added 2 ml distilled water and the contents were mixed. The homogenates were centrifuged (room temperature) at approximately 3,900 rpm (2,000 RCF) for 5 minutes (some homogenates from chinook salmon required 7 minutes), after which the supernatant liquid was decanted and discarded. The pellet was suspended in 0.5-0.8 ml (depending on size) of a solution containing 0.3M sucrose, 0.02M EDTA, O.lM imidazole, and 0.1% deoxycholate, pH 7.0 (room temperature) by thorough homogenization in a conical glass homogenizer (30 strokes). The suspension was centrifuged (room temperature) at 3,900 rpm (2,000 RCF) for 5 minutes. An appropriate volume of the resulting supernatant liquid was withdrawn, placed in a small test tube on ice, and then used as the enzyme preparation.

ATPASE DETERMINATION

Na+-K+ ATPase activity was calculated as the difference between activities observed in the presence and in the absence of ouabain. One set of reaction mixtures (0.65 ml in each test tube) consisted of (mM) $MgCl_2$, 23; NaCl, 115; CKl, 75; imidazole, 115; and ouabain, 0.58; adjusted to pH 7.0. A second set contained the same volume and reagents without ouabain.

Appropriate reagent blanks were included. All tubes containing the above solutions were placed in an ice water bath. Enzyme preparation (10 ml) was added to all reagent tubes followed by 0.1 ml of 0.03M Na_2ATP (adjusted to pH 7.0). The rack of tubes was then withdrawn from the ice bath, shaken, and placed in a constant temperature, circulating water bath at 37°C for 10 minutes. After the incubation period, the rack was again immersed in the ice bath. After reaction mixtures had thoroughly chilled, 0.1 ml 17.5% $HClO_4$ was quickly added to each tube, followed by 1.75 ml deionized water (room temperature) and 3.0 ml 2-octanol. All tubes were then placed in a specially constructed wooden rack to facilitate extraction. Without delay, 0.25 ml ammonium molybdate reagent was dispensed into each tube. The tubes were then covered with a sheet of plastic film. A wooden lid containing plastic foam was placed atop the plastic film (foam in contact with plastic film) such that pressure applied to the lid formed a seal over each tube. The rack of tubes was then shaken vigorously for 30 seconds to extract the phosphomolybdate complex into the octanol phase. The lid and plastic film were then removed and 0.5 ml citrate reagent added to each tube. A new sheet of plastic film was placed over the tubes and the extraction process repeated for 30 seconds. Molybdate reagent was prepared by adding 200 ml concentrated HCI to 58.4 g $(NH_4)_6MO_7O_{24}4.H_2O$ (ammonium molybdate), dissolving completely, then adding deionized water to 1 liter. The citrate reagent contained 143 g citric acid $(H_2C_6H_5O_7.H_2O)$ per liter, adjusted to pH 2.9 with NaOH (approximately 14 g).

Following the extraction procedure, all tubes were centrifuged briefly to facilitate complete separation of the aqueous and organic phases. The rack of tubes was then placed in a water bath (27O-28OC) to maintain constant temperature while determining absorbance. Aliquots of the octanol layer were withdrawn and read in a Beckman DB spectrophotometer at 312 mu (UV) against a reagent blank. Appropriate standards containing known quantities of phosphate were used to determine amounts of phosphate liberated from ATP during the reactions. Molar absorptivity of the phosphomolybdate complex under these conditions is 20,400. Protein concentrations in the enzyme preparations were determioned by the method of Lowry et al. (1951) as modified by Miller (1959), using bovine serum albumin as a standard. Each assay contained 25 ul of the enzyme preparation.

ATPase activities were calculated and expressed as micro-moles (urn) ATP hydrolyzed per mg protein per hour. Na+-K+ ATPase activity is the value determined in the reaction containing no ouabain minus the value obtained in the reaction with ouabain present.